WILDTYPE

Pre-Market Safety Consultation Summary

Prepared for the US Food and Drug Administration

June 2022

WILDTYPE

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1. Introduction

We believe that people who buy seafood should be able to understand exactly where their food comes from and how it was made. One of Wildtype's core values is transparency; it is one of the reasons we were the only company to respond to FDA's request for information on seafood labeling with product data that included nutritional comparability, organoleptic performance, and even the DNA identity of our salmon.

In that same spirit, we have engaged in a pre-market consultation with the U.S. Food and Drug Administration (FDA) and are hereby making our safety assessment public. The pre-market consultation process has entailed sharing with FDA all inputs used in the creation of our products and engaging in an iterative process of data sharing. The consultation also included our conduct of a hazard analysis of our end-to-end process, which is described in detail in this document. The goal of this analysis was to anticipate any potential hazards at each stage of production and identify preventive controls to mitigate these potential hazards.

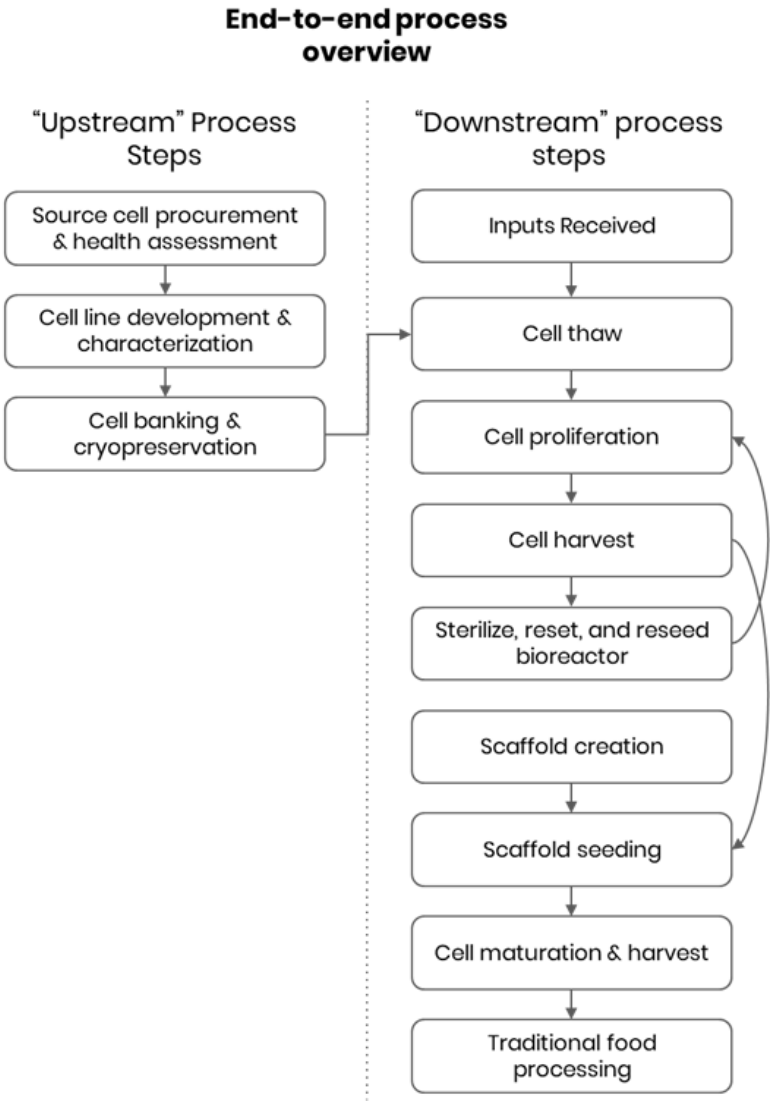
This document summarizes Wildtype's assessment of the safety of its seafood product based on our consultation with FDA. Below we present an overview of production technology, including cell growth and harvest. This is followed by a summary of our analysis of hazards and corresponding controls, and an assessment of the inputs used in production.

2. Technology overview

This document begins with an overview of Wildtype's production technology, ranging from seafood cell line development and characterization through final product harvest. For purposes of this pre-market consultation, It may be helpful to consider Wildtype's end-to-end process in two categories: an "upstream" set of activities comprising the initial cell line development steps, and a "downstream" set of activities ranging from thawing frozen cell lines to the harvest and packaging of the final product (Figure 1).

This bifurcation is a helpful construct because material inputs from the upstream activities other than fish cells are not present in Wildtype's products. Therefore, the majority of this safety assessment focuses on downstream steps.

Figure 1: Wildtype production process overview



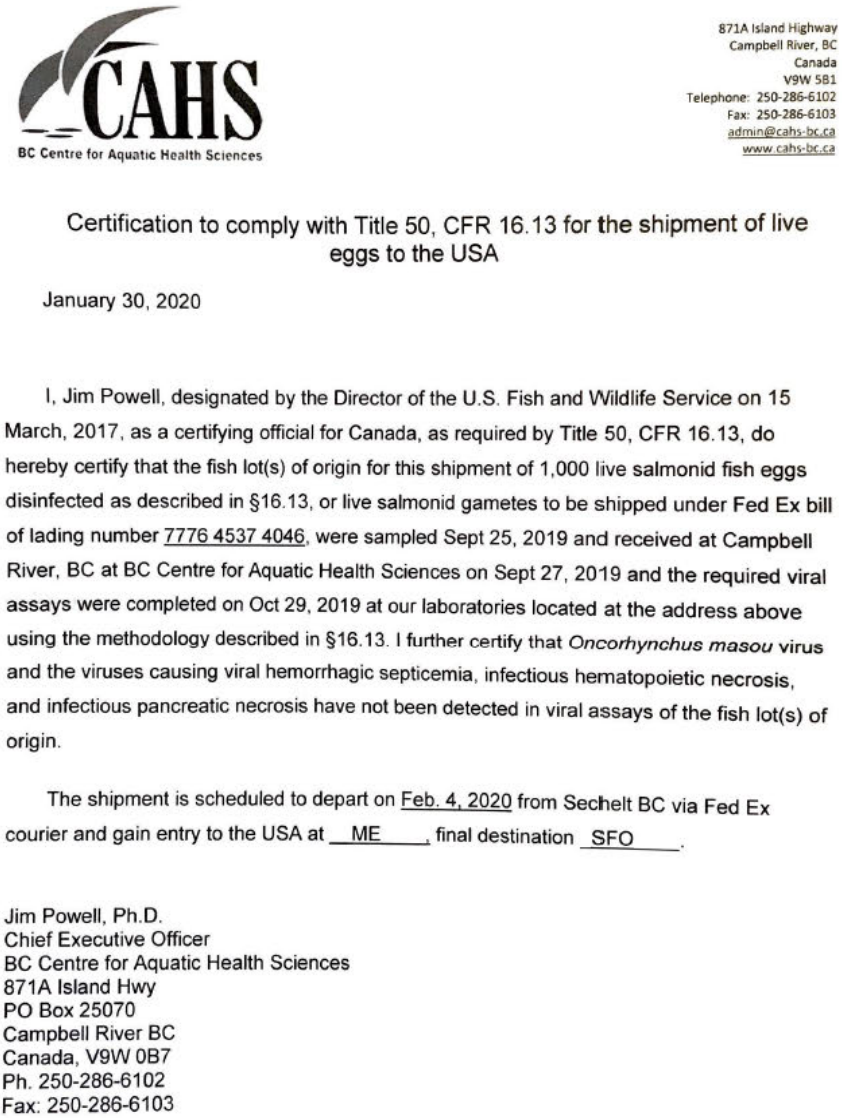
As illustrated in Figure 1, Wildtype's process begins with source cell procurement, in this case from salmon. Health assessments of the donor animals are completed and cell line development work commences. The process is largely characterized by trial-and-error attempts to identify cell strains that can sustain large-scale cell culture. Cells are banked and cryopreserved using traditional master/working cell bank practices. Cell proliferation occurs in custom-designed stainless steel cultivators (bioreactors) using a proprietary cell nutrient blend. Growth occurs without microcarriers, as Wildtype's production cell line has been adapted to grow in clusters, spheroids, or single-cell suspension. Cells are harvested from bioreactors and seeded on plant-based scaffolds developed by Wildtype. The final product is then rinsed, quality checked, and then subjected to conventional food processing and packaging steps. The remainder of this section describes each of the above steps in additional detail.

2.1 Upstream: source cell procurement and health assessment

Wildtype has developed its salmon cell lines from egg, alevin, and fry stages of development. All required federal and state import authorization was secured prior to receiving the fish at Wildtype's headquarters in California.

For example, all fish being imported into the United States from Canada are subject to a health assessment by a Canadian Fish and Wildlife inspector, validating that the fish are healthy and free of infectious disease. Similarly, US salmon hatcheries regularly evaluate the health of egg and juvenile fish, and do not release fish to Wildtype without first completing a virology assessment. Figure 2 provides a sample health certification for a shipment to Wildtype.

Figure 2 - Health certification completed prior to importing salmon eggs to the United States



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Certification to comply with Title 50, CFR 16.13 for the shipment of live eggs to the USA

January 30, 2020

I, Jim Powell, designated by the Director of the U.S. Fish and Wildlife Service on 15 March, 2017, as a certifying official for Canada, as required by Title 50, CFR 16.13, do hereby certify that the fish lot(s) of origin for this shipment of 1,000 live salmonid fish eggs disinfected as described in §16.13, or live salmonid gametes to be shipped under Fed Ex bill of lading number 7776 4537 4046, were sampled Sept 25, 2019 and received at Campbell River, BC at BC Centre for Aquatic Health Sciences on Sept 27, 2019 and the required viral assays were completed on Oct 29, 2019 at our laboratories located at the address above using the methodology described in §16.13. I further certify that *Oncorhynchus masou* virus and the viruses causing viral hemorrhagic septicemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis have not been detected in viral assays of the fish lot(s) of origin.

The shipment is scheduled to depart on Feb. 4, 2020 from Sechelt BC via Fed Ex courier and gain entry to the USA at ME, final destination SFO.

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Visual inspection of all fish or eggs is completed prior to cell isolation, which is performed under aseptic conditions. Microorganism contamination is controlled in initial passaging of cells via antifungal and antibiotic inputs added into the cell culture media. These inputs are not used in subsequent cell culture. Mass balance calculations showed that antibiotic amounts in the final product would not exceed $4.5 \times 10^{-68,636}$ ng/ml (Section 3.5) and are thus not further considered as a part of Wildtype's final product.

General discussion of potential contaminants in fish

The population of microflora associated with living fish reflects the microflora of their environment, modified by the microorganism's ability to multiply on sub-environments of skin, gills and GI tract.[1,2] Microflora of living fish from temperate and cold waters include *Psychrobacter, Moraxella, Pseudomonas, Acinetobacter, Shewanella, Flavobacterium, Cytophaga, Vibrio, Corynebacterium and Micrococcus* species. In fresh water, *Aeromonas* replaces *Vibrio* species. These potential hazards are mitigated in Wildtype's context by isolating source cells under sterile conditions and monitoring cell cultures for microbial contamination. Cell cultures affected by microbial contamination are terminated. Any potential microflora contamination of cell culture would quickly overtake the culture and be easily identified (both visually and with resultant fish cell death). In the rare case that one of these adventitious agents is able to propagate in cell culture, Wildtype's standard procedures call for the immediate sterilization and discarding of these cultures. These controls and systems have resulted in cell cultures that are invariably free of adventitious agents.

Potential bacterial pathogens to humans that are associated with finfish include *Clostridium botulinum*, various *Vibrio* species, *Plesiomonas shigelloides* and *Aeromonas hydrophila*.[3] The significance in human illness of the last two remains unclear. *Vibrio parahaemolyticus* can be found in fish; however, it is most common in warm waters, and virtually absent in cold water. *V. vulnificus* is associated with estuarial bottom feeders and *V. cholera is associated* with outbreaks. *Listeria monocytogenes* can be found in fish close to land, but is generally a contaminant from food processing environments. *Clostridium perfringens, Campylobacter jejuni, Yersinia enterocolitica, E. coli, Shigella spp*, and *Salmonella spp* when present in fish are generally from sewage pollution or terrestrial run-off. *Salmonella spp* risks in food production facilities stem largely from human handling and would be relevant to Wildtype's production technology. *Clostridium botulinum* is frequently found in aquatic sediment. As mentioned above, *Aeromonas spp* including *A. hydrophila* are common aquatic microbes and can cause enteritis in healthy hosts and more serious illness in the immunocompromised. *Plesiomonas shigelloides* (Vibrio-like organism) is a potential agent of diarrhea. Muscle tissue and internal organs of fresh caught, healthy finfish are normally sterile but bacteria can be found on skin, gills and GI tract. Bacteria on eggs would reflect the source fish and

[1] Sofos, J.N., Flick, G., Nychas, G-J., O'Brien, C.A., Ricke, S.C. and Crandall, P.G. 2013. Meat, Poultry, and Seafood. Chap. 6. In Doyle, M.P. and Buchanan, R.L. Eds. Food Microbiology: Fundamentals and Frontiers. 4th ed. ASM Press, Washington, D.C. pp. 111-167.
[2] International Commission on Microbiological Specifications for Foods (ICMFS). 2005. Fish and fish Products. Chap. 3. Microorganisms in Foods Vol. 6. Microbial Ecology of Food Commodities 2nd Ed. Kluwer Academic/Plenum Pub, NY. pp. 174-249.
[3] Ibid.

surrounding waters.[4] In the case of isolation from fish tissue (i.e. muscle biopsy), specimens are treated with hydrogen peroxide and ethanol for decontamination to eradicate bacterial contaminants prior to fish cell isolation and culture. When performing cell isolation from eggs, the risk of bacterial contamination is mitigated with hydrogen peroxide treatment before dechorionation and cell extraction.

Viruses of human concern (Noroviruses and Hepatitis A) are predominantly found in shellfish and arise from contaminated water or human handling.[5] These hazards are not relevant to Wildtype's production system for several reasons. First, municipal water is used as a starting source, which is subject to EPA-regulated viral decontamination strategies and confers an exceedingly low initial risk of these viral contaminations.[6,7] Second, direct human contact with water is not part of Wildtype's production methodologies, including all relevant processes of sterilization

Parasites (in colder waters tapeworms [*Diphyllobothrium*] and roundworms [*Anisakis* and *Pseudoterranova*]) are most frequent. *Anisakis simplex* is found in a variety of marine fish, including salmon, but is not found in farmed fish, as the parasites originate in the live feed consumed by wild fish.[8] *Trematodes* (liver flukes—*Opisthochis* and *Clonorchis*) and lung flukes—(*Paragonimus*) and intestinal flukes (*Heterophyidae* and *Echinostomatidae*) are typical of warm water areas. The risk of propagating parasites is obviated by isolating cells from fertilized eggs and growing them under aseptic conditions; the lifecycle of such parasites involves transmission to humans from a paratenic host (such as salmon) when these parasites are in the larval stage. Because egg hatching and larval maturation require an aquatic host's gastrointestinal system,[9, 10] parasite propagation does not proceed within cell culture and is not a concern for human transmission. Protozoans (*Cryptosporidium parvum*, *Cyclospora* and *Giardia*) are mainly transmitted by contaminated waters.[11] No cases from fish are known. Aquatic toxins and *Ciguatera* are not associated with salmon. Although these protozoa and parasites are not relevant to the company's production processes for the reasons described above, water used for all aspects of Wildtype's production process also undergoes sterile filtration (pore size = 0.2 m) to ensure absolute sterility.

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[4] International Commission on Microbiological Specifications for Foods (ICMFS). 2005. Fish and fish Products. Chap. 3. Microorganisms in Foods Vol. 6. Microbial Ecology of Food Commodities 2nd Ed. Kluwer Academic/Plenum Pub, NY. pp. 174-249.

[5] Ibid.

[6] Barrett CE, Pape BJ, Benedict KM, et al. Impact of Public Health Interventions on Drinking Water–Associated Outbreaks of Hepatitis A — United States, 1971–2017. MMWR Morb Mortal Wkly Rep 2019;68:766–770. DOI: http://dx.doi.org/10.15585/mmwr.mm6835a4

[7] Maunula L, Miettinen IT, von Bonsdorff CH. Norovirus outbreaks from drinking water. Emerg Infect Dis. 2005 Nov;11(11):1716-21. doi: 10.3201/eid1111.050487. PMID: 16318723; PMCID: PMC3367355.

[8] International Commission on Microbiological Specifications for Foods (ICMFS). 2005. Fish and fish Products. Chap. 3. Microorganisms in Foods Vol. 6. Microbial Ecology of Food Commodities 2nd Ed. Kluwer Academic/Plenum Pub, NY. pp. 174-249.

[9] Butt AA, Aldridge KE, Sanders CV. Infections related to the ingestion of seafood. Part II: parasitic infections and food safety. Lancet Infect Dis. 2004 May;4(5):294-300. doi: 10.1016/S1473-3099(04)01005-9. Erratum in: Lancet Infect Dis. 2005 Feb;5(2):81. PMID: 15120346.

[10] Kuchta R, Oros M, Ferguson J, Scholz T. Diphyllobothrium nihonkaiense Tapeworm Larvae in Salmon from North America. Emerg Infect Dis. 2017;23(2):351-353. doi:10.3201/eid2302.161026

[11] International Commission on Microbiological Specifications for Foods (ICMFS). 2005. Fish and fish Products. Chap. 3. Microorganisms in Foods Vol. 6. Microbial Ecology of Food Commodities 2nd Ed. Kluwer Academic/Plenum Pub, NY. pp. 174-249.

The risk of prion transmission is mitigated in Wildtype's approach by isolating cells from skeletal muscle tissue in cells rather than locations where prions are commonly found such as brain, spinal cord, lymph tissues, gut, etc.[12] The nature of prion transmission is also one that lends itself to a self-limiting course; fish prions are transmitted between cells[13] and overtake the closed system of cell culture, precluding the requisite expansion of cells for production.

Non-infectious adventitious agents are also potential sources of human toxicity when primary cell isolation and cell line development are performed from fish tissue; these toxins include arsenic and mercury.[14] These compounds, in addition to metals such as cadmium, can accumulate to toxic levels in the human body when consumed, and can result in a range of pathologic conditions. Any potential contamination of the starting material (i.e. primary cells isolated from fish) becomes successively diluted in each production run. Given that the starting material in each case is healthy, naturally occurring salmon, and that Wildtype's production processes do not contribute such adventitious agents to the final product, Wildtype's products have lower levels of these toxins than conventional salmon; these calculations are detailed in Section 3.5, and have been validated by third party laboratory testing as noted below.

Figure 3 – Metal contaminants in cultivated vs. conventional salmon

Compound	Result for cultivated salmon cells alone	Result for cultivated salmon (cells + scaffold)	Result for cultivated salmon cell culture media	Result for conventional salmon (wild king salmon)	Method	Acceptable level for conventional salmon
Arsenic	<50 ppb	<50 ppb	<50 ppb	**73 ppb**	ICP-MS	< 50 ppb
Mercury	<20 ppb	<20 ppb	<20 ppb	**36 ppb**	ICP-MS	< 20 ppb
Cadmium	<20 ppb	<20 ppb	<20 ppb	<20 ppb	ICP-MS	< 20 ppb
Lead	<100 ppb	<100 ppb	<100 ppb	<100 ppb	ICP-MS	< 100 ppb

Representative third-party testing conducted in March 2022 for adventitious agents in Wildtype's product (cultivated salmon cells alone and after scaffold integration [finished product]). These were compared to conventional salmon (wild king salmon in the above analysis). All methods used are validated for the intended purpose.

[12] Gough, K. C., & Maddison, B. C. (2010). Prion transmission: prion excretion and occurrence in the environment. *Prion*, 4(4), 275-282.
[13] Edward Málaga-Trillo, Evgenia Salta, Antonio Figueras, Cynthia Panagiotidis, Theodoros Sklaviadis, Fish models in prion biology: Underwater issues, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1812, Issue 3, 2011, Pages 402-414, ISSN 0925-4439, https://doi.org/10.1016/j.bbadis.2010.09.013.
[14] Ole Jakob Nøstbakken, Helge T. Hove, Arne Duinker, Anne-Katrine Lundebye, Marc H.G. Berntssen, Rita Hannisdal, Bjørn Tore Lunestad, Amund Maage, Lise Madsen, Bente E. Torstensen, Kåre Julshamn, Contaminant levels in Norwegian farmed Atlantic salmon (Salmo salar) in the 13-year period from 1999 to 2011, Environment International, Volume 74, 2015, Pages 274-280, ISSN 0160-4120, https://doi.org/10.1016/j.envint.2014.10.008.

Discussion of potential pathogens in food processing relevant to Wildtype's technology

In summary, while a number of potential pathogens are present in various marine species, most of these pathogens do not pose a risk in the context of Wildtype's production technology. The table below summarizes the principal pathogens of concern for Wildtype's technology. These pathogens and preventive controls are discussed in detail in Section 3.

Figure 4 – Summary of potential pathogens relevant to Wildtype's production systems

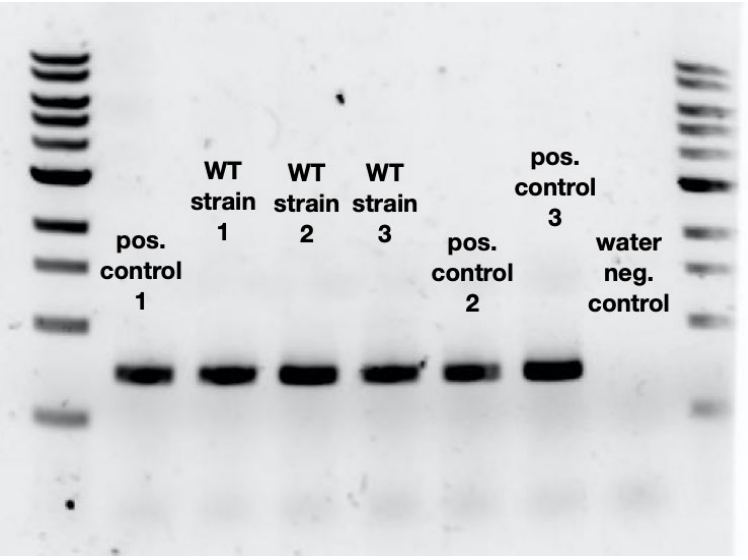
Potential pathogen	Potential source of contamination	Result for cells alone	Result for cell culture media	Result for the finished product (cells + scaffold)	Result for conventional salmon (farmed Atlantic salmon)	Detection limit (LOD)	Method
Listeria monocytogenes	Environmental and human handling	Not detected per 25g	Not detected per 25g	Not detected per 25g	**Detected per 25g**	0 / 25g	Real-time PCR
Salmonella spp.	Human handling	Not detected per 25g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	0 / 25g	Real-time PCR
Staphylococcus aureus	Human handling	<10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	10 cfu/g	Culture (non-chromogenic media)

Representative third-party testing for infectious contaminants in Wildtype's products. All methods used are validated for the intended purpose. Test date: 22 March, 2022. Note: cfu = colony-forming units.

2.2 Upstream: cell line establishment and characterization

Once initial cell isolation is complete, cells are propagated to assess long-term proliferation potential. During the research and development process, this occurs in dishes and flasks. Wildtype evaluates several cell line attributes, including proliferation rate, cell viability, differentiation potential, and ability to transition to 3-dimensional or non-adherent growth conditions. Potential cell lines are characterized visually to document cell morphology and by quantitative and semi-quantitative methods, including gel electrophoresis. Figure 5 below confirms that the DNA in various Wildtype cell lines is unequivocally Coho salmon DNA. This analysis was completed using genetic barcoding / confirmation by cytochrome C oxidase I.

Figure 5 - genetic barcoding: Wildtype cell lines vs. conventional salmon



Reverse transcriptase PCR using genomic DNA as a template from three of Wildtype's various Coho cell lines. Bands were sequenced and aligned to the Barcode of Life Database for unequivocal confirmation as Coho lines.

The musculature of embryonic or juvenile fish is used for cell isolation. Given that the target cell characteristics in the final product are those of muscle, fat, or connective tissue, isolated cells are first selected by attachment proclivities (i.e. affinity for structural proteins such as laminin, fibronectin, gelatin, etc.) and ability to thrive in various nutrient formulations. These attachment affinities and nutritional requirements predispose cells to have the capability of becoming muscle, fat, and connective tissues.

These cells are then characterized with respect to general shape (cellular morphology), proliferative capacity, genetic stability over the course of multiple generations, and gene expression patterns. The latter is used to confirm that these cells are of mesenchymal lineage (from which muscle, fat, and connective tissue develop).

2.3 Upstream: cell line development

No directed genetic engineering (i.e. gene editing) was used in the development of Wildtype's cell lines. To date, these cell lines have been generated using approaches that mine the intrinsic heterogeneity of cellular characteristics; this has been accomplished solely by standard selection methodologies, using the attachment affinities and nutritional conditions described in Section 2.2. Given the innocuous nature of this approach, no safety considerations or potential hazards were identified during cell line development. To validate this assessment, cell lines developed at Wildtype are nonetheless regularly subjected to 3rd-party testing for both microbiological and toxicologic contamination, which is further detailed in Section 3.

2.4 Upstream: cell banking and cryopreservation

Wildtype's production cell line has a two-tiered cell banking strategy comprised of master cell banks (MCB) and working cell banks (WCB). The company maintains three lots of its primary production cell line designated as MCB and two lots designated as WCB.

Cell banking procedures consist of seeding cells from a single source for expansion. Upon reaching confluence, cells are washed and centrifuged. The cell pellet resuspended in a cryopreservant. Aliquots are then transferred into vials, labeled with cell line name, operator name, date, and then cryopreserved in liquid nitrogen. Cell bank records are securely maintained.

Lots are periodically tested for bacterial (aerobic and anaerobic microorganisms such as *Staphylococcus spp*. and *Fusobacterium*, respectively), as well as fungal contamination (such as *Aspergillus*) that may be introduced by cell culture operators at this stage. This testing is completed by third-party testing agencies. These tests are always conducted prior to submitting any vials to Wildtype's master cell bank. See example test results from 3rd-party testing service in figure 6 below. *Mycoplasma* screens (also discussed in detail in section 3.2 below) are conducted prior to introducing new master cell bank cryovials. Mitigation of these contaminants is conducted primarily through aseptic technique described in detail in section 3.2 below. If contamination is detected at the cell banking or any other stage in Wildtype's process, cultures are terminated and deviation is noted in batch records. Investigation into the cause is initiated and appropriate corrective actions are enacted, where appropriate.

Figure 6: example sterility test results

Specimen Description

Species: fish

Description: Cells

Number of Specimens/Animals: 1

Client ID	Investigator	Species
CM	Carissa	Fish

Services/Tests Performed: Cell Line Sterility Testing 1 + Anaerobic Culture (1)

Microbiologic evaluation for: Aerobic bacterial growth, Anaerobic bacterial growth, Fungal growth

Summary: All test results were negative.

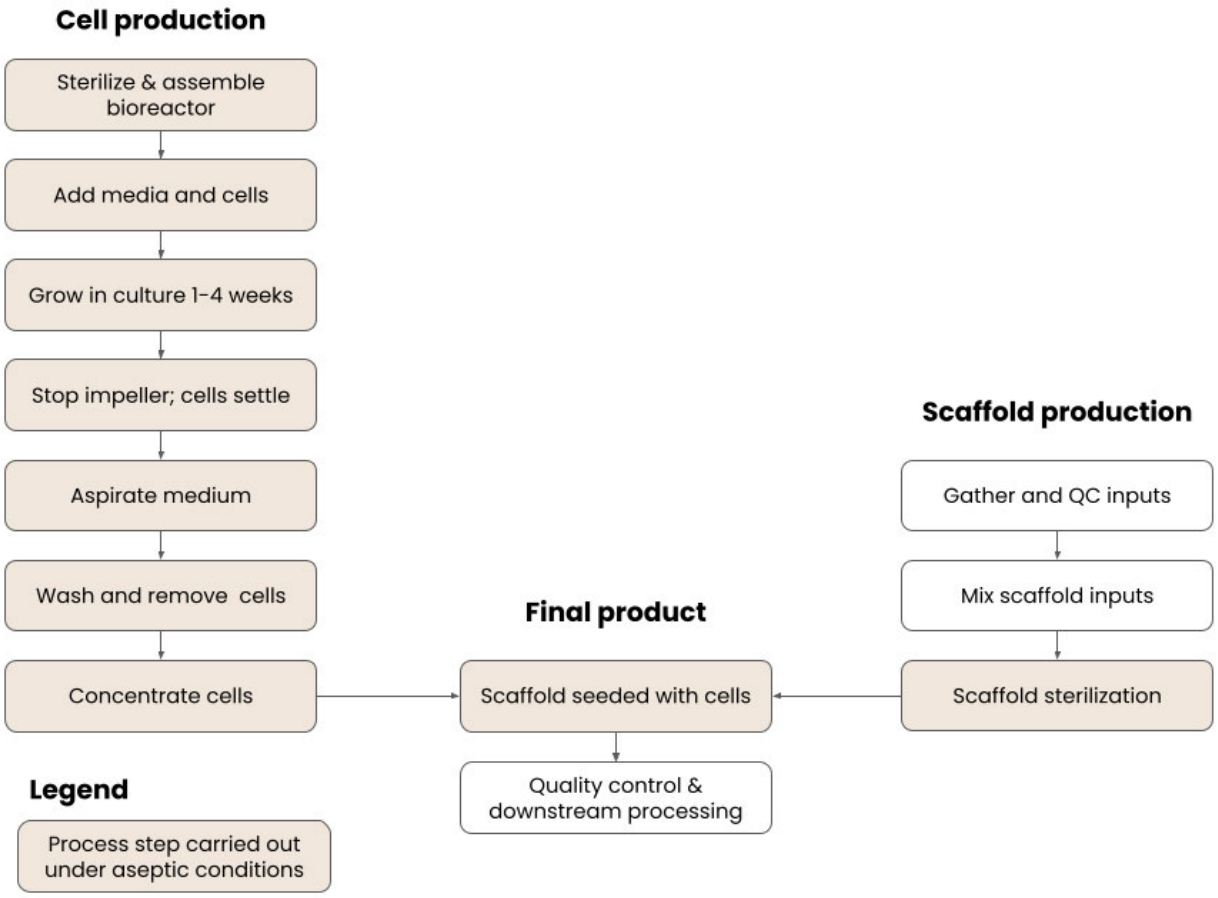
MCBs are stored in two separate geographic locations. Additionally, Wildtype stores MCB vials at its offsite, out-of-state backup storage facility as a safety precaution for natural disasters.

Production vials of a single species are housed in a dedicated liquid nitrogen tank to prevent cross-contamination between species as well as with vials that are the subject of active research and development. Sufficient labeling is applied to prevent potential errors.

Wildtype has also completed DNA and RNA sequencing analyses of its cell lines for a complete characterization of all expressed genes.

The remainder of this section introduces Wildtype's downstream production steps. Detailed discussion of potential hazards and preventive controls follows in the next section. The above steps comprise non-recurring R&D activities. Subsequent steps associated with production, and therefore the focus of this safety assessment, are depicted in figure 7 below.

Figure 7: Wildtype production process



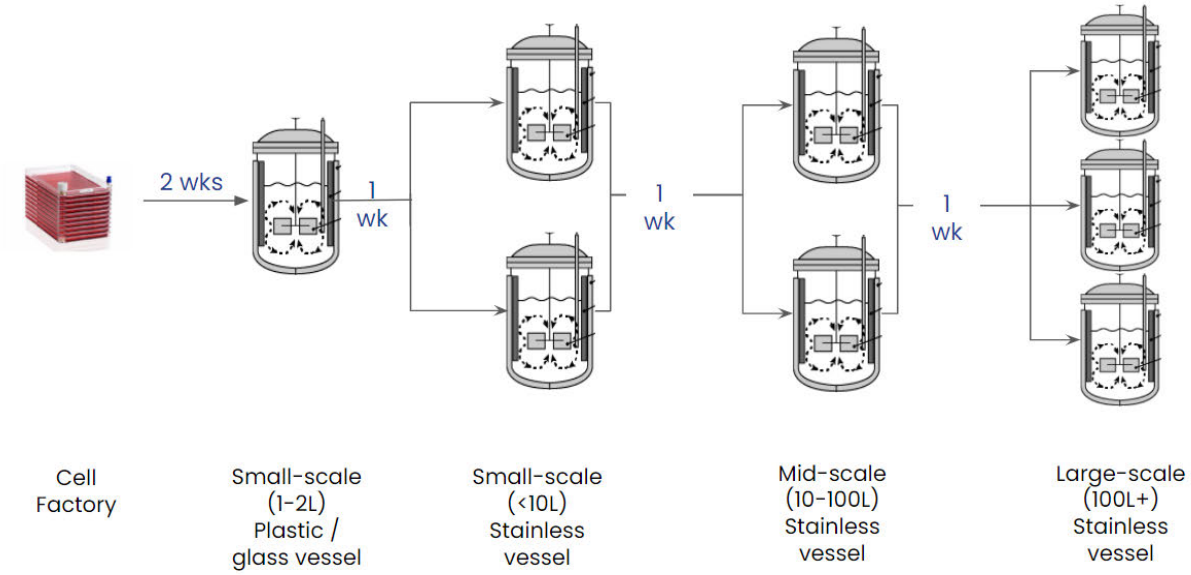
2.5 Downstream: inputs received

Upon receipt of inputs needed for the downstream production process, such as powdered cell culture media and scaffold inputs, Wildtype's production staff validates contents and quality attributes, including aerobic plate counts. Containers showing signs of tampering are rejected. Production inputs are transferred to clean, dry storage spaces until they are used for the production steps described below.

2.6 Downstream: cell thaw & proliferation

The cell proliferation stage begins by thawing a vial of cells from the working cell bank. As previously noted, Wildtype's primary production salmon cell lines grow in cultivators (bioreactors) without the use of microcarriers. The company's production seed train uses multi-layered plates ("cell factories") or agitated flasks to initiate cultures. As figure 8 below illustrates, cells from these small-scale flasks are used to seed larger, fully-enclosed stainless steel vessels, which are in turn used to seed larger stainless steel vessels.

Figure 8: Illustrative 3-dimensional seed train overview



Wildtype uses custom steel bioreactors that were designed for its suspension cell cultures. Although customizations were made to parameters like impeller design, height : diameter aspect ratio, and port design, Wildtype's bioreactors resemble stainless steel vessels currently used in both fermentation and cell culture at industrial scale.

2.7 Downstream: cell harvest

After one to four weeks of growth in a bioreactor, cells are harvested. Agitation in the bioreactor ceases and cells are allowed to settle. Supernatant is removed from the tank using peristaltic pumps. Cells are then centrifuged, collected, and quality tested.

At the completion of a batch, the bioreactor is taken apart, sterilized using a combination of clean-in-place, steam-in-place, and clean-out-of-place techniques, reassembled, tested, and prepared for the next run.

2.8 Downstream: scaffold production, cell seeding and maturation

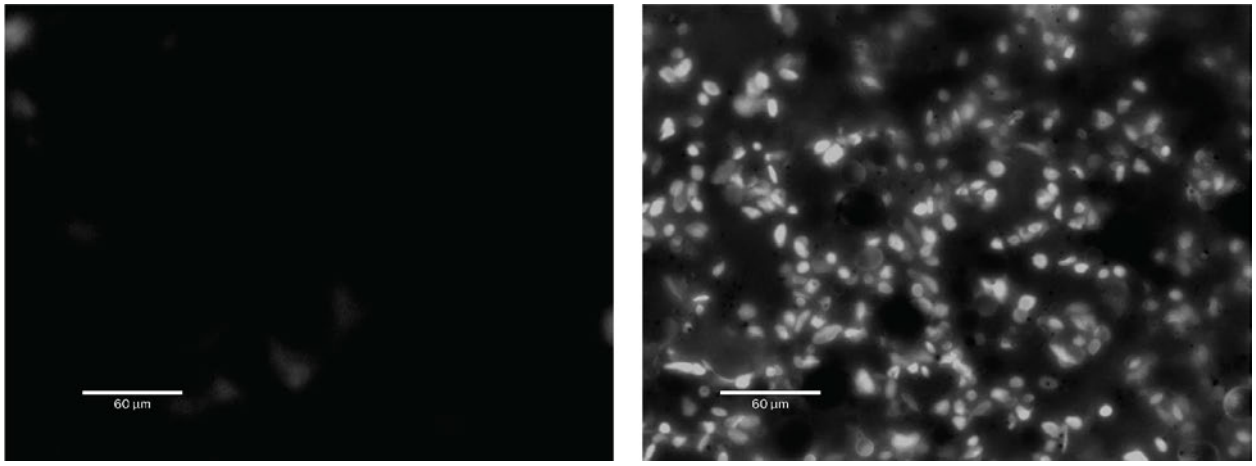
The propagation and harvest of cells is not sufficient to enable the development of complex 3-dimensional structures; for that, structural guides known as scaffolds are used. Scaffolds are comprised of compounds that are similar to those naturally found between cells; they provide a framework that enables cells to grow, mature, and organize naturally.

Wildtype's scaffolds are made using a blend of plant-based proteins, sugars, and fats, all of which are present in the US food supply today. Those inputs are used in a manner consistent with their existing regulatory status (e.g. Generally Recognized as Safe [GRAS]). Scaffold components are selected according to these criteria because they are incompletely degraded, and therefore present in the final product.

Inputs for Wildtype's scaffolds are gathered by operators, quality-checked, mixed, and assembled. Inputs are then sterilized using techniques such as heating or ethanol treatment, and are seeded with cells under aseptic conditions. The process of maturation and differentiation is reliant upon the scaffold components and structure; for example, cells display characteristics of muscle when seeded on parallel scaffold fibers (such as fiber formation, expression of myogenic genes, and multinucleation), and take on characteristics of fat when seeded on scaffolds with a structure and fat content that is conducive to this transformation. This process can take several weeks, depending upon the desired textural and nutritional attributes. Upon completion of the maturation / differentiation process, the product is taken through a lethal thermal process prior to being subjected to further traditional food processing steps.

Figure 9 demonstrates DNA staining (4',6-diamidino-2-phenylindole [DAPI]) on scaffold alone prior to seeding (left panel) and the final cultivated salmon product (right panel) to illustrate the cellular density and uniformity within cultivated salmon.

Figure 9 - Fluorescent staining of salmon cell nuclei within cultivated salmon



As a guiding principle, the company has refrained from even testing potential inputs if they are not currently present in the American food supply, with a use that mirrors the company's intended use.

2.9 Downstream: traditional food processing steps

After the seeded scaffold has undergone cell integration, it is visually inspected for defects. After this point, the product is packaged and moves through conventional food processing steps, including a quality control inspection for microorganisms. Figure 10 below shows a sample of Wildtype's finished product in a sushi form factor.

Figure 10 - Wildtype's cultivated salmon nigiri (March 2021)

Figure 11 below summarizes interim analytical data and measurement methods employed for Wildtype's ready-to-eat Pacific salmon product as of April 2022. All methods are validated for the intended purpose, and testing was completed on 18 April, 2022. Wildtype expects the product specifications below to more closely approximate conventional salmon by the time Wildtype salmon is available for commercial sale in the United States.

Figure 11 – Interim analytical data: Wildtype pacific salmon (April 2022)

Component	Result	Method
Kilocalories	134 kcal / 100g	Calculation
Moisture and volatiles	79.5%	Vacuum oven
Carbohydrates, calculated	4.39%	Calculation
Cholesterol	7.2mg / 100g	GC-FID
Total omega-3 isomers	2.83%	GC-FID
Total omega-6 isomers	1.11%	GC-FID
Total omega-9 isomers	3.92%	GC-FID
C22:6 Docosahexaenoic acid (DHA)	1.45	GC-FID
C20:5 Eicosapentaenoic acid (EPA)	0.88	GC-FID
Total saturated fats	2.4%	GC-FID
Total trans fatty acids	<0.02%	GC-FID
Crude fat by acid hydrolysis	11.33%	Acid hydrolysis
Ash	0.69%	Combustion
Protein	4.38%	Combustion
Iron	0.0007%	ICP-OES
Potassium	0.058%	ICP-OES
Sodium	0.122%	ICP-OES
Calcium	0.006%	ICP-OES
Folate / folic acid	0.062mg / 100g	LC-UV/DAD and Nephelometry
Vitamin B1 / thiamine hydrochloride	0.016mg / 100g	Spectroscopy
Vitamin B12	3.76 g / 100g	Nephelometry
Pantothenic acid / B5	0.059mg / 100g	LC-UV/VIS
Beta-carotene	281 IU / 100g	LC-FAD-DLD
Retinol	<30 IU / 100g	LC-FAD-DLD
Total vitamin A	281 IU / 100g	LC-FAD-DLD
Zinc	1.9 ppm	ICP-OES

3. Safety Assessment

Wildtype has conducted an end-to-end hazard analysis of its production process and has identified a number of preventive controls that it has already implemented or is in the process of implementing. The section below focuses on the “downstream” production steps.

3.1 Summary: hazard analysis and preventive controls

Figure 12 below outlines the potential hazards and preventive controls envisioned for each step of Wildtype’s production process. Note that this is a risk assessment that is in progress as production steps may be further defined as processes evolve during scale-up.

Figure 12: Hazard analysis and preventive controls

(1) Ingredient / Processing Step	(2) Identify potential food safety hazards introduced, controlled, or enhanced at this step B biological C chemical P physical		(3) Do any potential food safety hazards require preventive control?		(4) Justify your decision for column 3	(5) What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *Process including CCPs, Allergen, Sanitation, Supply chain, other preventive control*	(6) Is the preventive control applied at this step?	
			Yes	No			Yes	No
Upstream process steps: Source cell procurement, cell line establishment, and cryobanking	B	Potential presence of microflora[15] or bacterial[16] pathogens, viruses[17], and parasites[18] in donor animals during cell isolation		X	Microflora, bacterial, and viral contamination mitigated through aseptic technique & subsequent lethal step Parasite introduction mitigated by isolating cells from eggs and via sterile filtration			
	C	None						
	P	None						

[15] Potential microflora of concern include general *Psychrobacter, Moraxella, Pseudomonas, Acinetobacter, Shewanella, Flavobacterium, Cytophaga, Aeromonas, Corynebacterium* and *Micrococcus* - please refer to broader discussion of these hazards on page 6.
[16] Potential bacteria of concern include *Clostridium botulinum*, various *Vibrio* species, *Plesiomonas shigelloides, Listeria monocytogenes,* and *Aeromonas hydrophila* - refer to page 6
[17] Potential viruses of concern include Noroviruses and Hepatitis A - see additional discussion on pages 6-7
[18] Potential parasites of concern include tapeworms, roundworms, Anisakis simplex, liver flukes, lung flukes, intestinal flukes, and Protozoans - please refer to page 7 for additional detail

(1)	(2)		(3)		(4)	(5)	(6)	
Inputs received	B	Potential pathogens in scaffold inputs[19]		X	Subsequent lethal step; supplier approval program			
	C	Potential for undeclared allergens in media or scaffold inputs	X		Potential for suppliers to include undeclared allergens by cross-contact	**Supply chain preventive control:** Supplier approval process and verification by audit	X	
	P	Potential packing materials in inputs	X		Damaged packaging or shipping materials may contaminate inputs	**Process preventive control**: Visual inspection of all packages; random X-ray sampling of inputs	X	
Cell thaw	B	Potential pathogens[20] from environment or human contact		X	Pathogens would outcompete cell growth- controlled by monitoring for contamination on each batch Subsequent lethal step			
	C	Potential introduction of non-labeled allergens	X		Possibility of thawing incorrect vial in multi-species cryobank	**Process Preventive Control:** Dedicated cryobank for each species; staff double checks each vial for correct species	X	
	C	Potential for unapproved food additives (freezing agents) in product		X	Mass balance calculations show that freezing agents not present in final product Analysis of finished product shows absence of freezing agents			
	P	None						

[19] Potential pathogens of concern include *Salmonella, Listeria monocytogenes, and Staphylococcus aureus*
[20] Potential pathogens of concern include *Salmonella, Listeria monocytogenes, and Staphylococcus aureus*

(1)	(2)		(3)		(4)	(5)	(6)	
Seed train & cell proliferation	B	Potential growth of pathogens[21] in cell culture from environment or human contact	X		GMP risk reduction: Aseptic technique by trained employees. Pathogens would outcompete cell growth; monitoring for contamination on each batch (via pH monitoring) Critical limit for pH set to 7.8 Hygienic conditions: Documented monitoring of critical CIP/SIP (temperature, pH, etc.) parameters. CIP/SIP records will be part of production batch records. Subsequent lethal step	**Process Preventive Control**: Cultures that experience a pH above 7.8 or below 7.1 are determined to be at risk for contamination and subjected to further screening including microscopy.	X	
	C	Clean-in-place chemical residue may be present in bioreactors		X	Validated CIP for equipment with documented monitoring of amounts of chemicals used. CIP records will be part of production batch records			
	P	Potential for metal fragments	X		Metal-to-metal contact can produce metal fragments	**Process Preventive Control**: X-Ray (conducted in a subsequent step)		X

[21] Potential pathogens of concern include *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus*

(1)		(2)	(3)		(4)	(5)	(6)	
Cell harvest	B	Potential growth of pathogens[22]	X		Pathogens, if present, may grow in harvest, however, process is short and GMPs used throughout	**Process Preventive Control**: Thermal process in subsequent step		X
	C	None						
	P	Potential for metal fragments	X		Metal-to-metal contact can produce metal fragments	**Process Preventive Control**: X-Ray (conducted in a subsequent step)		X
Scaffold production & seeding	B	Potential introduction of pathogens from inputs[23]		X	Supply chain approval program for ingredient suppliers including COA; Subsequent lethal step; Hygienic conditions in this area			
	C	Potential unlabeled seafood or plant allergens present in product	X		Seafood is present in product Allergenic plant-based inputs are used as an input to WT's scaffolds	**Allergen preventive control:** Ensure all allergens are properly labeled (conducted in subsequent step)		X
	C	Potential introduction of unapproved chemicals in inputs		X	Supply chain approval program for ingredient suppliers including CoA Letter of guarantee from suppliers			
	P	Potential for metal fragments	X		Metal-to-metal contact can produce metal fragments	**Process Preventive Control**: X-Ray (conducted in a subsequent step)		X
	P	Potential for hair or articles of jewelry to fall into product		X	GMP production practices are implemented including gowning and hair/beard nets			

[22] Potential pathogens of concern include *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus*
[23] Potential pathogens of concern include *Salmonella, Listeria monocytogenes, and Staphylococcus aureus*

(1)		(2)	(3)		(4)	(5)	(6)	
Cell maturation	B	Survival of potential pathogens[24]	X		Pathogens may survive this process step if thermal process is insufficient	**Process Preventive Control:** Product is heated to lethal temperature	X	
	B	Potential spore forming organisms[25] during cooling		X	Blast chiller reduces temperature out of the danger zone (26 - 54.5˚C) to 4˚C in <90 minutes	Validated cooling process quick enough to preclude spore formers from growth.		
	B	Potential introduction of pathogens by environment and worker contact[26]	X		Environmental pathogens can recontaminate during handling if contact surfaces not properly cleaned	**Sanitation Preventive Control:** EMP and hygienic zoning	X	
	C	None						
	P	Potential introduction of metal fragments in finished product	X		Metal-to-metal contact can produce metal fragments	**Process Preventive Control**: Metal detector & X-Ray (conducted in a subsequent step)		X

[24] Potential pathogens of concern include *Salmonella, Listeria monocytogenes, and Staphylococcus aureus*
[25] Potential pathogens of concern include *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus*
[26] Potential pathogens of concern include *Salmonella*, Listeria *monocytogenes,* and *Staphylococcus aureus*

(1)		(2)	(3)		(4)	(5)	(6)	
Post-production processing	B	Pathogens introduced by environment and worker contact during packaging[27]	X		Environmental pathogens can recontaminate during handling if contact surfaces not properly cleaned	**Sanitation Preventive Control:** EMP and hygienic zoning	X	
	C	Potential presence of undeclared allergens in final product	X		Label producer may not include required allergen disclosures	**Allergen Preventive Control:** Double check during label design and receiving that label contains required allergen notices	X	
	P	Non-detection of metal fragments in finished product	X		An improperly operating X-Ray instrument may miss potential metal fragments.	**Process Preventive Control**: X-Ray step prior to storage, shipping, and distribution	X	

[27] Potential pathogens of concern include *Salmonella, Listeria monocytogenes, and Staphylococcus aureus*

3.2 Discussion: hazard analysis and preventive controls

Given the novelty of this means of seafood production, Wildtype partnered with Dr. Marcos Sánchez-Plata and Samuel Peabody of Texas Tech University to conduct a thorough hazard analysis and preventive control design. Professor Sánchez is an Associate Professor in Animal and Food Sciences with research focus areas of risk assessment and performance evaluation of integrated food safety management systems, in-plant validation of antimicrobial interventions, and other topics related to food security. He is one of the first academic researchers to explore the topic of food safety as it applies to cell-cultivated meat and seafood production. Wildtype would like to acknowledge Dr. Sánchez and Mr. Peabody for their support during this process.

General Discussion of Adventitious Agent Mitigation

Microorganisms such as bacteria, fungi, parasites, and others may be introduced inadvertently into a manufacturing process for food. The growth parameters for propagating cells are permissive for growth of microorganisms. By virtue of relying on cell culture for a critical input in its manufacturing process, Wildtype maintains sterile technique through each process step described in figure 7, with the exception of scaffold production and post-harvest processing.

Sterile technique alone significantly mitigates the risk of microbial contamination in the production process. Regular pH and visual monitoring during cell production also provides early warning of any microbiological contamination; visual checks take two forms: gross inspection and microscopy. Gross inspection occurs every time a culture is sampled or transferred, and constitutes inspection for turbidity that would represent a microbial overgrowth in the culture. Samples are also observed under a microscope when cultures are samples and when irregularities (such as slowed culture growth) are observed. As discussed above, sampled of Wildtype cells are periodically sent to external laboratories for microorganism testing to confirm sterility.

Mycoplasma represent a common and insidious contaminant of cell cultures, as they are able to pass through sterility filters and are not always visible under common light microscopy.[28] For these reasons, screens are periodically conducted on Wildtype cell lines to ensure that production and R&D lines are free from contamination; these screens are performed with a frequency based on standards in cell culture and whenever contaminations are suspected (notably, when proliferation rate slows or when cell morphology changes are observed). Testing is conducted by fluorescent staining and microscopy and PCR, as is standard practice.[29] To date, Wildtype has never had a *Mycoplasma* positive test.

[28] Young L, Sung J, Stacey G, Masters JR. Detection of Mycoplasma in cell cultures. Nat Protoc. 2010 May;5(5):929-34. doi: 10.1038/nprot.2010.43. Epub 2010 Apr 22. PMID: 20431538.
[29] Ibid.

The risk of these adventitious agents is further mitigated by strict adherence to standard aseptic cell culture techniques[30] throughout the upstream process. Good Manufacturing Practices (GMPs) are also employed, including biosafety cabinet usage, hand washing, glove utilization, material and personnel movement, and entry protocols. Although the range of precautions / operating procedures encompassed by these aseptic techniques is broad, the salient principle is that of continuously safeguarding cultures from microbial exposure. This involves transferring cell cultures between vessels in aseptic environments (i.e., high efficiency particulate air [HEPA]-filtered laminar hoods with sterilized surfaces) and periodic monitoring for potential contaminants. It also involves the use of equipment, such as pipettes and sampling devices, which are sterilized by standard practices such as autoclave or gamma irradiation.[31] Wildtype's monitoring system includes pH monitoring, irregularities of which portend bacterial infection, microscopic inspection for infectious contamination, and testing of samples (both plating studies and polymerase chain reaction [PCR] screening for known contaminants). Deviations from Wildtype's standard aseptic operating procedures are documented and investigated to ensure the integrity of cultures are not compromised at any point during production. Finally, cultures suspected of harboring adventitious contamination are discontinued to prevent the propagation of pathogenic agents. The growth of pathogens is mitigated in Wildtype's production system via aseptic technique and the termination of cultures contaminated with bacteria.

Upstream Process Steps

As previously discussed, inputs used in upstream process steps, with the exception of cells, are not present in Wildtype's finished product. For completeness however, we address potential hazards in the source cell procurement, cell line establishment, and cryobanking steps described in Figure 1.

One potential hazard was identified during the upstream process steps, namely the potential for bacterial pathogens or viral / parasite transmission from the donor animal into cell culture. A detailed discussion of typical pathogens associated with fish is discussed on pages 6-8. Specific pathogens relevant to Wildtype's production technology are described in Figure 4. Microorganism transmission is mitigated through the use of aseptic technique. As discussed in detail below, microorganisms would quickly outcompete our aquatic cell lines and hence rigorous sterile technique is required. Viruses of concern primarily stem from shellfish and are therefore not relevant for Wildtype's initial products. Finally, the potential for parasite transmission is mitigated by isolating cells at the egg stage of development and maintaining aseptic technique, including sterile filtration with a pore size of 0.2µm, throughout cell line establishment activities.

[30] Coté RJ. Aseptic technique for cell culture. Curr Protoc Cell Biol. 2001 May;Chapter 1:Unit 1.3. doi: 10.1002/0471143030.cb0103s00. PMID: 18228291.

[31] Marin Berovic, Sterilisation in biotechnology, Biotechnology Annual Review, Elsevier, Volume 11, 2005, Pages 257-279, ISSN 1387-2656, ISBN 9780444519528, https://doi.org/10.1016/S1387-2656(05)11008-4.

Inputs Received

Three potential hazards were identified during the process of receiving material inputs for Wildtype's production process. First, potential pathogens including *Salmonella, Listeria monocytogenes, and Staphylococcus aureus* are recognized as potentially present within the inputs received from suppliers. Inputs for cell culture media and inputs used in scaffolds are received from different classes of suppliers. Cell culture media inputs are sourced from suppliers accustomed to supplying the bio-pharmaceutical industry with tightly controlled quality standards. Inputs from food suppliers are not subject to the same quality controls, and Wildtype has designed its supply chain preventive controls accordingly.

This potential hazard did not require a preventive control as there is a lethal thermal step at the end of Wildtype's production process that eliminates these potential pathogens. Further, Wildtype maintains a supplier approval program that is discussed below. Finally, good manufacturing practices and hygienic controls in place throughout Wildtype's process mitigate the risk of potential contamination propagating to subsequent production steps.

Second, there is the potential for undeclared allergens to be present in inputs received from suppliers due to cross-contact in suppliers' facilities. In this case, a supply-chain preventive control was deemed appropriate, whereby Wildtype will maintain a supplier approval program and will verify supplier specifications via routine audits using a risk-based monitoring system. In addition to audits of our scaffold input and other critical suppliers, upon selecting a new supplier of scaffold materials, Wildtype will carry out an in-house allergen test to identify the presence of unintended allergens in the materials. Formal quality agreements will be maintained with all critical suppliers which will detail responsibilities of the supplier for certain quality activities including allergen prevention and reporting of deviations to Wildtype. Additionally, Wildtype will carry out random allergen testing of scaffold inputs at least twice a year, or when routine testing suggests potential issues or risks.

Third, rigid packaging materials such as wood, Styrofoam, straps, and plastic may infiltrate inputs in the event that crating and other shipping materials are crushed or damaged in transit. Wildtype maintains a process preventive control at this point that requires receivers to document any visible damage to input packaging upon receipt. Packages that are damaged or not secure are visually inspected by operations staff for any potential infiltration of packaging materials. Finally, random lots of inputs are periodically scanned using X-ray to ensure the absence of foreign materials in Wildtype's input shipments.

Cell thaw

Wildtype's downstream process begins in earnest with the thawing of working cell banks to commence seed trains and cell growth. Three potential safety hazards were identified at this stage. First, as cells are thawed from vials and plated on cell culture plates or flasks, there is a potential risk for growth of the pathogens described above. A preventive control was not deemed necessary at this stage due to the presence of several GMP process controls. First, aseptic technique is used for all handling of cells. This includes the use of laminar flow hoods

or biosafety cabinets to minimize the risk of contamination. All relevant employees are trained prior to handling cells and undergo annual re-training. Additionally, probes monitor the pH of cell cultures in real time, rapid changes to which almost invariably indicate the growth of adventitious agents.

More specifically, the inherent proliferative advantage of most microbial contaminants (with doubling times as fast as 20 minutes, compared with doubling times on the order of days for Wildtype's aquatic species cell lines) results in swift pH fluctuations that often thwart cell line propagation when such microbes are inadvertently introduced into the production system. For this reason, pH monitoring is considered a first-line methodology for the safeguarding of production systems. During the cell thaw stage, pH is monitored using a standard indicator, such as phenol red. During all subsequent stages of production, from seed train, cell proliferation, and harvest, pH is monitored using pH probes in bioreactors that monitor and record pH on a continual basis. The target pH is 7.4, and critical pH limits of 7.1 and 7.8 have been set to alert bioreactor operators that the culture is out of range.

Inspection of suspected contaminations, either by direct microscopy or with optical density probes that continuously evaluate the turbidity of cultures, provide further indications of microbial growth. A process preventive control is in place at this stage and at all subsequent cell culture steps (seed train, cell proliferation, and cell harvest), whereby contaminated cultures are terminated and the process starts anew. Further, a subsequent lethal step in the process mitigates this potential hazard. We expect our finished products to contain no heavy metals above established limits and any microbiological contaminants to be within limits acceptable for conventional seafood, which are detailed in Figure 3.

Second, Wildtype works with several types of seafood, each with a unique allergen profile. If cryopreserved vials of different seafood cells were stored together in the same liquid nitrogen freezers, an operator could potentially start a seed train with an unintended cell line. Wildtype adheres to the industry-standard master and working bank described above in section 2.4. This includes labeling vials and maintaining accurate working and master cell bank records. A process preventive control is applied at this step and requires a separate liquid nitrogen dewar for each seafood species. The purpose of this preventive control is to limit the potential for an operator to erringly thaw the incorrect species of seafood and put that into production, potentially introducing an unanticipated allergen. The control at this step requires two individuals to be present when removing a vial from the cryobanks. Two written certifications validate that the dewar and vial species match and that they are the correct species for production. Seed train operators are required to review this documentation and sign off that the target species matches what was thawed before moving cell lines into production.

A third potential hazard is the inadvertent introduction of cryopreservant into Wildtype's products. Mass balance calculations were performed for cryopreservation agents to demonstrate that these inputs would not be present in the final product. Additionally, periodic random checks are performed on the final product by gas chromatography-mass spectrometry (GC-MS) to ensure freezing agents are not present in the finished product.

No physical hazards were identified at this stage.

Seed train and cell proliferation

Three potential hazards were identified at this process step. First, potential pathogens of concern – *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* – could grow in cell culture. A preventive control for this risk was not deemed necessary due to several GMP processes in place: aseptic technique conducted by trained employees and real-time pH monitoring that would indicate the presence of pathogenic growth. Additionally, clean-in-place (CIP) and steam-in-place (SIP) records will be included in production batch records. Finally, a lethal step at the conclusion of Wildtype's production process mitigates this potential hazard.

Second, Wildtype's bioreactors are sterilized using CIP, SIP, or a combination of both methods. A potential hazard was identified whereby clean-in-place chemical residues may remain in bioreactors after cleaning. Preventive controls were not deemed necessary to address this potential hazard due to two process GMP steps. Sterilization standard operating procedures (SSOPs) are in place for Wildtype's CIP process. SSOP validation confirms that no chemical residues are present at the completion of the CIP process. All CIP SSOPs will be re-validated if significant changes are made to equipment or other aspects of the production process. Additionally, Wildtype limits its use of clean-in-place agents to permissible chemicals widely used in the food and beverage industry in the United States. All CIP monitoring records will be included in the production batch records for each lot of products.

A third potential hazard at this stage includes metal fragments produced by metal-to-metal contact in the final product. Wildtype's bioreactors are made from stainless steel and include stainless steel mechanical agitators. A process preventive control during the post-harvest processing stage entails the use of X-ray to identify metal and other adventitious fragments in the final product prior to shipping to customers.

Cell harvest

Two potential hazards were identified at this stage: growth of pathogens during cell culture and metal fragments from metal-to-metal contact. Both of these potential hazards are discussed above.

Scaffold production and seeding

Wildtype's scaffold production step includes several activities including gathering ingredients, mixing, shaping, the seeding of cells, and a lethal thermal step. Five potential hazards were identified during these steps.

First, the pathogens described above might be introduced through scaffold ingredients, environmental exposure,[32] or manual handling. As discussed above, this potential hazard is mitigated through a supply chain approval program, a subsequent lethal step, and hygienic conditions in the area in which scaffolds are prepared.

Second, unlabeled seafood or plant allergens could be present in the final product if labels were inaccurately produced. An allergen preventive control is in place at a later stage of production.

Third, there is the potential for unapproved inputs to be used in scaffold inputs if suppliers made an error in fulfillment. A process preventive control was not deemed necessary to address this potential hazard, however, as Wildtype maintains a supply chain preventive control program for ingredient suppliers. Scaffold inputs must be accompanied by a valid certificate of analysis. A letter of guarantee is requested from suppliers providing inputs in high concentrations in Wildtype's scaffolds. Additionally, Wildtype will conduct annual audits with its suppliers that will focus on the three pathogens of concern discussed above: *Listeria, Salmonella*, and *Staphylococcus*. Wildtype's input suppliers will be required to produce results of a successful audit showing the absence of these pathogens on an annual basis.

Fourth, because mixing and handling equipment involves metal-to-metal contact, there is a potential for the introduction of metal fragments at this stage of production. A process preventive control for this potential hazard is introduced later in the production process.

Fifth, Wildtype's scaffold materials are currently assembled and put into an automated mixer manually. As such, typical physical hazards associated with food production apply to Wildtype's production process including loose jewelry and hair falling into mixing ingredients and equipment. Good manufacturing practices are in place for this step including required gowning, removal of loose jewelry, and donning of hair/beard nets prior to entering Wildtype's scaffold production area.

Cell maturation

The cell maturation step includes time to allow cells to mature, a lethal thermal step, cooling, and final product cutting and trimming. The process of cell maturation represents the integration of cells within a scaffold; in this stage, the scaffold serves to guide the 3-dimensional organization and proliferation of cells into the various structures that comprise the final product. The termination of this process is effected by a lethal thermal step, whereby the product is exposed to a temperature of 65˚C for 120 minutes. This step serves two important purposes: First, this lethal temperature halts all further maturation and growth of Wildtype's cell lines, ensuring production consistency. Second, it neutralizes pathogens that might be present, rendering the product free of living pathogens. The validation of this pathogenic eradication step occurs with routine testing of the final product for infectious and

[32]Pathogens of concern here include *Listeria monocytogenes*, *Salmonella* serovars, and *Staphylococcus*. All three are ubiquitous in food processing facilities. Given that Wildtype's product is classified as RTE and that the product is exposed to the environment prior to packaging, an environmental monitoring program is in place to mitigate risk of these pathogens.

toxin-mediated pathogenic agents. Four potential hazards were identified at this stage of production.

First, pathogenic contamination (*Listeria, Salmonella, Staphylococcus*) during cell culture is possible, as described above. Potential pathogens may survive the final thermal process if temperature and heating times are insufficient. A validated process preventive control is instituted at this step to heat the product to a lethal temperature.

A preliminary validation of this thermal step was completed in April 2022 in collaboration with Dr. Marcos Sánchez-Plata and Sam Peabody at Texas Tech University. FDA Guidelines recommend cooking finfish to a minimum internal temperature of 63ºC (145ºF).[33] However, no such guidance exists for cell-seeded finfish products. To study and characterize the appropriate conditions, Wildtype salmon was subjected to a thermal process post-packaging for at least one hour at 60ºC. A series of validation experiments was designed to quantify the survival of microbes in the packaged product. Cell-seeded salmon were inoculated with *Salmonella*, vacuum packaged, and treated to a water bath with controlled times and temperatures. *Salmonella* spp. were chosen for their resilience to heating. The *Salmonella* in the salmon were enumerated after treatment in the water bath. Three linear regression models were determined including their D-values.[34]

It was determined that at D60ºC a 12 log reduction could be achieved in as few as 8.8 minutes. As salmon are normally held at 60ºC temperature for 1 hour, the thermal processing step clearly eliminates any non-spore forming foodborne pathogens that are reasonably likely to enter such products via adventitious means.

Figure 13. Slope and D Values for Survival of *Salmonella* spp. in heat-treated products at constant temperatures.

Temperature (˚C)	Slope (log(CFU)/m)	Std error	D_1 value (m)	D_{12} value (m)
57.5	-0.3298815	0.0213728	3.031391575	36.37669891
60	-1.35795	0.113983	0.7364041386	8.836849663
62.5	-2.426315	0.09977273	0.4121476395	4.945771674

Second, potential germination and growth of spore-forming organisms is a risk during the cooling process if the scaffold remains in the danger zone of 26-54.5˚C. A preventive control is not implemented at this step because a blast chiller is used to reduce the temperature of the product to 4˚C in less than 90 minutes.

[33] Fish and Fishery Products Hazards and Controls Guidance – Fourth Edition, June 2021, accessed using this link.
[34] A similar experimental study was carried out in Alejandra Ramirez-Hernandez, Brenda Inestroza, Amy Parks, Mindy M Brashears, Marcos X. Sanchez-Plata, Alejandro Echeverry; Thermal Inactivation of *Salmonella* in High-Fat Rendering Meat Products. *J Food Prot* 1 January 2018; 81 (1): 54–58.

Third, any handling by personnel or exposure to the environment following the thermal process creates the potential for pathogenic contamination of the final product. A sanitation preventive control establishes an environmental monitoring program and hygienic zoning to control potential exposure to the pathogens described above. This part of the process occurs in a high-care area of Wildtype's production facility. The environmental monitoring program is described in detail in section 3.10 below.

Fourth, because metal surfaces are used during this stage of production, there is a potential for metal fragments to be present in the finished product. A process preventive control (X-ray) during a subsequent step addresses this potential hazard.

Post-production processing: labeling, packaging, metal detection, and storage

While post-harvest processing is not the focus of this safety assessment, several important potential hazards were identified and preventive controls implemented. Three potential hazards were identified during the post-harvest steps.

First, ready-to-eat products may become contaminated with the pathogens described above during packaging if contact surfaces are not properly cleaned or if they are exposed to the environment. A sanitation preventive control at this stage consists of an environmental monitoring program and hygienic zoning.

Second, the potential for undeclared allergens in the final product is possible if labels do not include allergen disclosures required by inputs used in previous process steps. An allergen preventive control during the labeling stage of production requires staff to double check that the label contains all required allergen disclosures both during the label design and label receiving steps. There is also a double-check process that will be documented in production batch records.

Third, metal fragments potentially introduced into the finished product from earlier processing stages may be missed if X-ray equipment is improperly operating. A process preventive control is in place at this final stage of production to use a validated X-ray process to detect the presence of potential metal fragments prior to storage, shipping, and distribution. Personnel will be trained regularly on the operation of the equipment and identification of possible malfunctions.

Wildtype is implementing an electronic document storage system that will maintain production batch records for each lot of finished products. This information will include quality assurance (QA) monitoring through all production steps. This will enable QA managers to review all relevant documentation to ensure that products meet Wildtype's quality and food safety criteria prior to release into distribution. This system will also allow Wildtype to trace products back to individual ingredient lots and forward into the distribution chain. The system will also track corrective actions required if specifications are not met.

3.3 Material input safety assessment

In addition to completing a hazard analysis and preventive control exercise, Wildtype undertook a comprehensive material input safety assessment. This assessment detailed the material inputs for each process step involved in Wildtype's technology from source cell procurement through final product harvest. It did not contemplate traditional food processing steps, as those steps are well understood.

Although product development activities are ongoing, most of the inputs examined in the analysis are not likely to change substantially. If the company introduces additional inputs that will be present in the first version of our commercialized products, additional safety assessments for these inputs will be completed.

3.4 Approach to safety assessment and material input overview

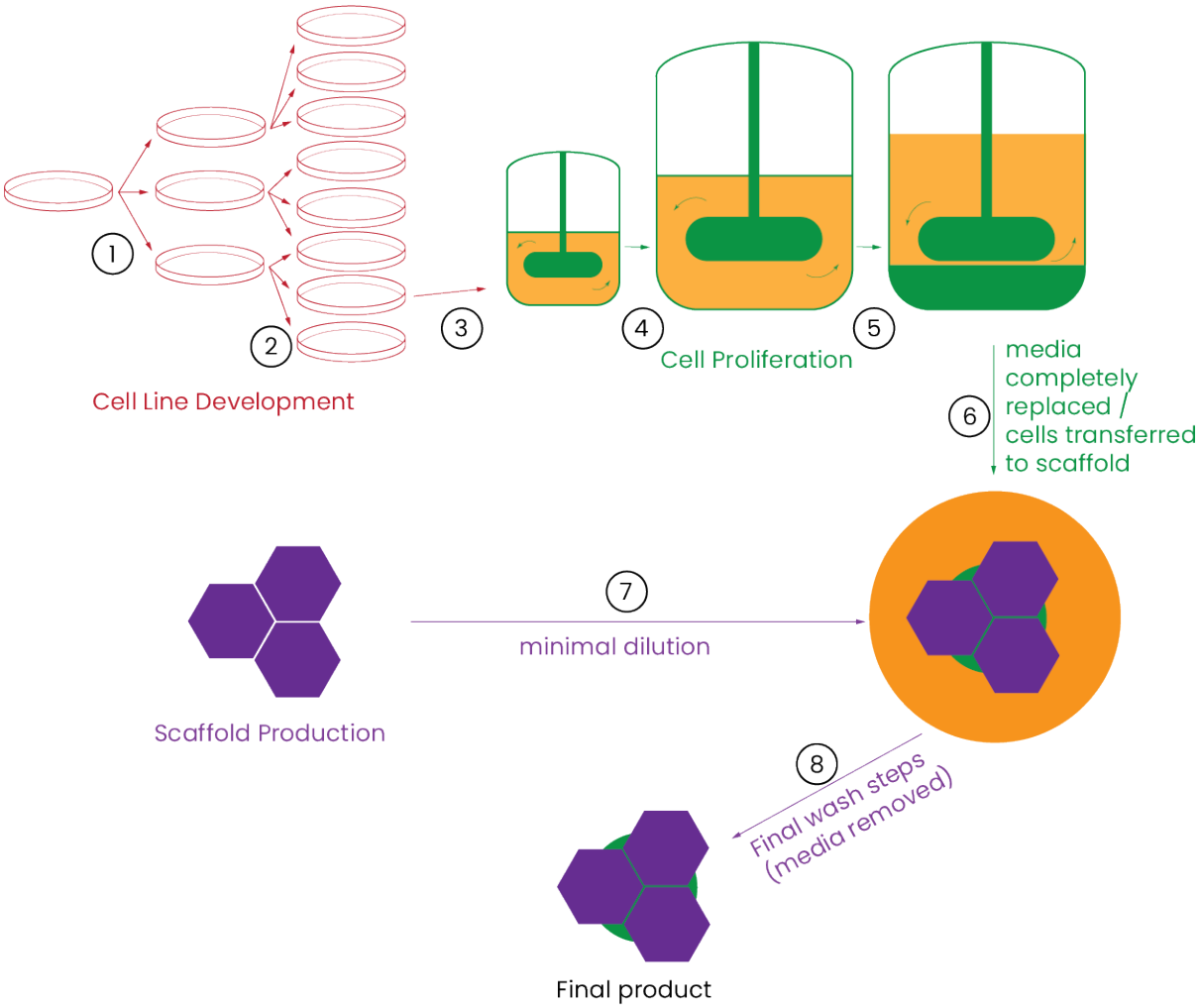
During the assessment process, 116 material inputs were identified including both upstream and downstream process steps. Of these, 20 inputs in our cell culture medium and feed are not present as ingredients in the existing food supply. It should be noted that many of these inputs are normal constituents and precursors of all food (e.g., DNA bases or energy sources). A safety analysis was conducted for each of these 20 inputs. Seven other media inputs were also examined, as the evidence of existence in the food supply was only tangentially related to Wildtype's use. For all 27 inputs, a calculation of expected concentration in the final product (via an estimated daily intake analysis) was completed to illustrate that these compounds would not be present in the finished product (Figure 14). As a secondary step, these EDI levels were compared to established safety thresholds (e.g., scientific literature or the Threshold for Toxicological Concern [TTC]). The conclusion of this analysis was that all 27 of these inputs either are not present in the final product at meaningful levels, or are present only inadvertently and at very low concentrations (often below the limit of detection). Sections 3.5 and 3.6 below describe the assumptions used to create Figure 14. A detailed safety assessment in section 3.7 discusses three specific inputs, nicotinamide adenine dinucleotide, fibroblast growth factor, and taurine (bolded in the table below) as representative cases illustrating the general point that the concentrations of these inputs are orders of magnitude below safety thresholds discussed in the scientific literature.

Figure 14 - Results of daily estimated intake safety assessment

Input	Predicted concentration in finished product (mg / L)	Estimated Daily Intake (mcg / kg bw / day)	Estimated Daily Intake (mcg / day)
pH indicator	1.70E-30	2.62E-30	1.85E-28
Serum	1E-14	1.54E-14	1.09E-12
Growth Factor/Hormone	5.5E-12	8.47E-15	6.00E-13
Freezing Agent	4E-31	6.16E-31	4.36E-29
Freezing Agent	5E-7156	7.7E-7156	5.45E-7154
Inorganic Salt	3E-11	4.62E-11	3.27E-09
Coenzyme	1.3E-12	2.00E-12	1.42E-10
Component of DNA	5.2E-12	8.01E-12	5.67E-10
Component of DNA	5.2E-12	8.01E-12	5.67E-10
Component of DNA	5.2E-12	8.01E-12	5.67E-10
Nicotinamide adenine dinucleotide	**5.2E-13**	**8.01E-13**	**5.67E-11**
Sugar	2E-12	3.08E-12	2.18E-10
Sugar	9.4E-13	1.45E-12	1.02E-10
Glucose metabolite	9.4E-13	1.45E-12	1.02E-10
DNA base	5E-14	7.70E-14	5.45E-12
Cofactor	3.6E-12	5.54E-12	3.92E-10
Cofactor	5.2E-13	8.01E-13	5.67E-11
Component of RNA	5.2E-13	8.01E-13	5.67E-11
Vitamin	3.1E-13	4.77E-13	3.38E-11
Vitamin	1.7E-13	2.62E-13	1.85E-11
Vitamin	5.2E-13	8.01E-13	5.67E-11
Fibroblast Growth Factor	**1E-15**	**1.54E-15**	**1.09E-13**
Selenium	6.7E-15	1.03E-14	7.3E-13
Taurine	**2.2E-12**	**3.39E-12**	**2.4E-10**
Polysorbate	6.5E-12	1E-11	7.1E-10
Antioxidant	1E-13	1.54E-13	1.1E-11

3.5 Mass balance assumptions and calculations

Figure 15: Wildtype process overview and safety approach



Cell Line Development is the process of cell isolation, characterization, and propagation for research purposes only. This is generally conducted in dishes and flasks (2-dimensional surfaces). From there, production-appropriate cell lines are identified, and transitioned to the Cell Proliferation phase. This is where cells are grown for human consumption; they are incorporated with Scaffolds (made of plant-derived inputs) and washed to remove residual nutrient media prior to packaging. Each step is detailed here, with calculations of relative ranges; Figure 15 above serves to demonstrate the sequential nature of these steps. These calculations were then used for each input in question in our material safety assessment.

As background, cells are grown in a nutrient formulation (media) from stages 1-6. This formulation contains all classes of ingredients that cells naturally require for growth, including fats, sugars, amino acids, minerals, and salts. The nutrient media is maintained at a pH that supports cell growth, and the vessels used for cell propagation are temperatures conducive to normal cell proliferation. As noted above, media inputs are naturally-occurring

components that cells use for energy (i.e. sugars and fats), to create new proteins (i.e. amino acids), to support normal metabolism (i.e. minerals and trace metals such as iron), et cetera. Of the inputs that are not present as stand-alone ingredients in the existing food supply, the analyses that follow demonstrate the low (and often undetectable) levels at which they are present in the final product. Importantly, because these components support normal cell growth, their presence contributes to the product's overall nutrition profile when consumed.

Results of a mass balance exercise described in detail below can be broken into three discrete categories of inputs. First, inputs used in steps 1 and 2 are non-recurring activities. Any inputs used at this stage are in practical terms not present in the finished product. Second, inputs used in steps 3-6 would be found in the finished product in concentrations between 3.27×10^{-9}µg per day to 5.45×10^{-7154}µg per day in the finished product and therefore not present in the final product at meaningful levels. Third, inputs used in steps 7 and 8 would be present in the finished product in detectable amounts, however, inputs in this category were limited to those already in active use in the US food supply, used in a manner consistent with Wildtype's use.

Assumptions for the concentrations of inputs found within cells (which apply to steps 1-6 and 8, prior to the washes that largely remove components not contained within cells) are as follows:

1) For elements, complex ions, and molecules that freely diffuse through the cell membrane, we adopt the most conservative assumption that the extracellular concentration achieves an equivalent equilibrium concentration within the cell. For example, an input with a concentration of 11mg/L in the cell culture medium results in a concentration of 11mg/L within the cell. Given the small sizes of these molecules, we assume free diffusion across the cell membrane along concentration gradients; in reality, this likely overestimates the intracellular concentration of the inputs, as free diffusion is often limited by a semipermeable lipid bilayer membrane.

2) For formed, functional proteins, we assume that internalization of these proteins results by classical endocytic pathways, which either leads to degradation of the protein or recycling back to the cell surface (either residing on the extracellular surface or exocytosed from the cell). In both cases, this results in a far lower concentration inside the cell than that found in the initial cell culture media. First, only proteins that bind receptors on the cell surface are the ones actively internalized. Second, the combination of either degradation or recycling as an endpoint for these proteins means their intracellular concentration is lower than that of the extracellular nutrient media. The literature around endocytosis of extracellular components is

extensive and spans decades. Three relevant reviews[35,36,37] describe this process in more detail.

Given the properties of these functional proteins (which include growth factors such as insulin-like growth factor [IGF], paracrine signaling molecules such as insulin, binding proteins such as albumin, and proteins involved in the intercellular transport of nutrients such as transferrin), this class of input deserves special consideration. The presence of these proteins is not only physiologic, but also generally required to sustain normal cellular growth and development. Additionally, cellular independence from such proteins is generally only observed in pathological states.

With respect to safety, functional proteins present in the US food system are first considered for direct comparison. In the case of fish, functional proteins (such as insulin-like growth factor-1 [IGF1], fibroblast growth factor-2 [FGF2], insulin, and others) are naturally present, and support normal growth and development as they do in other animal species.[38, 39] Wildtype's production process similarly uses these naturally-occurring proteins to sustain cellular growth and maturation; given that conventional seafood does not undergo safety screens that contemplate these proteins (and that no deleterious consequence of seafood consumption has implicated functional proteins, including allergic reactions described in section 3), the company references conventional fish for *de facto* established acceptable concentration ranges. In the case of coho salmon, for example, the serum concentration of IGF1 varies from 45-117 ng/ml, depending upon the stage of fish development.[40] The activity of rainbow trout fibroblast FGF2 has also been studied, demonstrating a similar peak activity within the range of 10-100 ng/ml, depending on how much heparin is present.[41]

Functional proteins are indispensable in normal physiology, and similarly sustain the growth of Wildtype's cell lines. During production, the concentration of each functional protein never exceeds those found in nature and, as noted above, is typically present in far lower concentrations in the final product on account of sequential washing steps.

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[36] Goh LK, Sorkin A. Endocytosis of receptor tyrosine kinases. *Cold Spring Harb Perspect Biol.* 2013 May 1;5(5):a017459. doi: 10.1101/cshperspect.a017459. PMID: 23637288; PMCID: PMC3632065.

[37] Hall, C., Yu, H. & Choi, E. Insulin receptor endocytosis in the pathophysiology of insulin resistance. *Exp Mol Med* 52, 911–920 (2020). https://doi.org/10.1038/s12276-020-0456-3

[38] Chandhini, S., Trumboo, B., Jose, S. *et al.* Insulin-like growth factor signalling and its significance as a biomarker in fish and shellfish research. *Fish Physiol Biochem* 47, 1011–1031 (2021). https://doi.org/10.1007/s10695-021-00961-6

[39] Dena M Leerberg, Rachel E Hopton, Bruce W Draper, Fibroblast Growth Factor Receptors Function Redundantly During Zebrafish Embryonic Development, *Genetics*, Volume 212, Issue 4, 1 August 2019, Pages 1301–1319, https://doi.org/10.1534/genetics.119.302345

[40] Munetaka Shimizu, Penny Swanson, Haruhisa Fukada, Akihiko Hara, Walton W. Dickhoff, Comparison of Extraction Methods and Assay Validation for Salmon Insulin-like Growth Factor-I Using Commercially Available Components, General and Comparative Endocrinology, Volume 119, Issue 1, 2000, Pages 26-36, ISSN 0016-6480, https://doi.org/10.1006/gcen.2000.7498

[41] Jun-ichiro Hata, Jiro Takeo, Shinya Yamashita, Heparin Essential for Tissue Culture with Fish Fibroblast Growth Factor 2, Fisheries science, 1998, Volume 64, Issue 2, Pages 216-219, Released June 30, 2008, Print ISSN 0919-9268, https://doi.org/10.2331/fishsci.64.216

3) Some molecules accumulate in the cell; the only examples of this from the inputs described above are precursors to normal cellular structures. Examples include amino acids, which are incorporated into molecules created by the cells (such as collagen), and DNA bases that become incorporated into nascent DNA as cells proliferate. The amount of DNA and other physiological compounds can be quantified in the final product, and are invariably equivalent to those of conventional seafood because they represent normal components of natural cellular structures. Of the inputs in our process, those that meaningfully accumulate as they are incorporated into cellular structures are amino acids, nucleic acid components, and fats.

Process steps (referenced in figure 10):

1. Cells are passaged (split and propagated) in a nutrient medium. When one input is present initially and then removed in the subsequent passaging step, the calculation is as follows:

[Starting concentration] x 0.002 x 0.002 {2 washing steps} x 0.003 {input} x 0.001 {resuspension} = 1.2×10^{-11} (with this repeated for each cell passage, typically ~50 before cells are moved to Cell Proliferation = $[1.2 \times 10^{-11}]^{50}$ = **1.2×10^{-550}**).

Rationale: In a 10mL culture, we use an example of an input that is present in the nutrient medium at a concentration of 100mM. The supernatant is aspirated from the cells, removing 99.9 % (10µL is a conservative estimate of the remaining medium volume in practice). This amount is washed with 5mL of buffered saline (diluting it by 10µL / 5000µL = 0.002) , bringing the new concentration to 0.002 x 100mM = 200nM. This is typically repeated again, further diluting the input by 0.002 (= 400pM). 3mL of the input solution is then added to detach the cells, further diluting the input by 10µL / 3000µL = 0.003 (= 1.3pM). The cells are then resuspended in 10mL of fresh media (approximately 10µL [cells] x 10000µL [media]) = a further dilution of 0.001.

Of the inputs under consideration, this applies to our cryopreservant alone.

2. Some compounds are added in early stages of Cell Line Development, but subsequently omitted; an example is an antibiotic cocktail that is used to ensure sterility of cultures initially, but is no longer used after passage #10.

For these inputs, the calculation is the same as above, except that, instead of the 50 passages assumed for Step 1, the input is present for the first 10 passages and is diluted over the next 40 passages. The equation now becomes:

[Starting concentration] x 0.002 x 0.002 {2 washing steps} x 0.003 {input} x 0.001 {resuspension} = 1.2×10^{-11} (with this repeated for each cell passage, typically ~40 before cells are moved to Cell Proliferation = $[1.2 \times 10^{-11}]^{40}$ = **1.2×10^{-440}**).

3. The transition from Cell Line Development to Cell Proliferation involves eliminating certain inputs from the culture, for example, an input used to indicate pH. Here, the same starting equation above is used first:

[Starting concentration] x 0.002 x 0.002 {2 washing steps} x 0.003 {input} x 0.001 {resuspension} = 1.2×10^{-11}

In this case, however, the cells are not resuspended into 10mL as a final step; instead, they are resuspended in a 1L bioreactor. Therefore, the equation now becomes:

[Starting concentration] x 0.002 x 0.002 {2 washing steps} x 0.003 {input} x 0.00001 {resuspension} = **1.2×10^{-13}**

4. There is dilution that occurs during scale-up between bioreactors, for which the calculation is as follows:

[Starting concentration] **x 0.0005**

Rationale: In this example, a 1L bioreactor will be scaled to a 10L bioreactor. Cells are centrifuged, and the supernatant is removed as a first step. Assuming the cell pellet is 5g per 1L culture, this is approximately a 5mL pellet by volume, which is then resuspended in the new volume of 10L (a 0.0005 dilution)

5. Dilution of inputs also occurs when the nutrient medium is exchanged. Typically, approximately of the media is exchanged in a particular run. Although this can happen several times as the culture is propagated, we assume this only occurs once for a more conservative estimation of dilution. Therefore, the calculation for this step is simply:

[Starting concentration] **x 0.5**

6. When cells are harvested and seeded onto a scaffold, the nutrient media formulation is completely replaced with new media. The calculation is as follows:

[Starting concentration] **x 0.005**

Rationale: Again assuming a cell mass of 5g per 1L culture, the cells are centrifuged and the supernatant is removed; as a conservative estimate, we will assume that the cells are then seeded on a similar volume of scaffold / nutrient medium. Therefore 5g (5ml) of cells is effectively resuspended in 1000ml, resulting in a dilution of 0.005.

7. The scaffold is seeded with cells; this does not result in significant dilution of scaffold input concentrations.

8. The final product is washed three times with buffered saline solution; this does not introduce new inputs, and only serves to dilute the nutrient media. The cells and scaffold remain undiluted. The dilution of nutrient media input applies to most media components, and is calculated as follows:

[Starting concentration] x 0.0001 x 0.0001 x 0.0001 {3 washes} = **1×10^{-12}**.

Rationale: For this calculation, we assume the final washes are performed for a 100g product (which consists of cells, scaffold, and nutrient media). As noted above we can only assume that the nutrient media constituents are diluted; the cells and scaffold remain intact. In 100g of product, we can assume that < 10% is free nutrient media (we will use the 10% upper limit, and assume that 10ml is free nutrient media). This is removed, with a likely residual media volume of 50µl. This is then washed with 500ml, resulting in a dilution of 0.0001. This wash step is repeated twice (3 total washes), resulting in a dilution of 1×10^{-12}.

3.6 Summary: estimated daily intake calculations

To assess the presence of the aforementioned 27 inputs in the final product, an estimated daily intake (EDI) was calculated for each input. The EDI was based on salmon consumption derived from the food consumption data collected in the National Health and Nutrition Examination Survey (NHANES) 2013-2016. The highest 90th percentile 2-day average daily salmon intake, on a gram per day basis, was 112g/day among adolescents 13-18y (see Figure 19) and the highest 90th percentile on grams per kilogram of body weight basis was 2.56g/kg bw/day among children 2-12 years old. Detailed information on the data, method, and results of salmon intake assessment using the NHANES are provided below. For the purpose of this safety assessment, the highest 90th percentile salmon intake was combined with the predicted concentrations to calculate the EDIs. All calculated EDIs are vanishingly small and consumer exposure to these substances is not anticipated.

The conclusion of the estimated daily intake exercise was that the 27 inputs in question ranged from 3.27×10^{-9} µg per day to 5.45×10^{-7154} µg per day in the finished product and therefore not present in the final product at meaningful levels.

Furthermore, all calculated EDIs were well below the lowest threshold of toxicological concern (TTC), which is 0.15µg/day (structural alert for genotoxicity) as described in the recent comprehensive review by EFSA and the WHO (2016).[42] Figure 16 provides the TTC values summarized in the 2016 report by the EFSA and WHO. Based on the 2016 EFSA/WHO comprehensive review, the TTC approach is a valid screening tool, based on scientific risk assessment principles, to assess low dose chemical exposures, and to distinguish those for which further data are required to assess the human health risk from those with no appreciable risk. Given that all the EDIs are well below the most conservative TTC value, it can be concluded that there is no safety concern. We acknowledge that this framework cannot be applied to all inputs under consideration, and is being included as a secondary point of reference to the EDI calculations described above. The inclusion of the TTC framework here is intended only to demonstrate the exceptionally low levels at which the 27 inputs in question are present in the final product.

[42] European Food Safety Authority (EFSA) and World Health Organization (WHO), 2016. Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree;; PUBLISHED: 10 March 2016

Figure 16 - TTC values[43]

Type of TTC value	TTC value in μg / person per day
With structural alert for genotoxicity	0.15
OPs and carbamates	18
Cramer Class III	90
Cramer Class II	540
Cramer Class I	1800

Salmon Consumption

Salmon intake was derived from food consumption records collected in the What We Eat in America (WWEIA) component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2013-2014 and 2015-2016 (NHANES 2013-2016). NHANES is a continuous survey that uses a complex multistage probability sample designed to be representative of the civilian United States (U.S.) population (NCHS 2018, 2016). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection and non-response.

NHANES 24-hour dietary recall

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 14,601 individuals in the survey period 2013-2016 provided 2 complete days of dietary recalls. Only individuals that provided two reliable dietary recalls were included in this analysis.

Identification of salmon in the diet

This consumption analysis identified foods reported consumed in the WWEIA, NHANES 2013-2016 that corresponded to salmon and foods that contained salmon as an ingredient. The selection of foods was based on USDA's Food and Nutrient Database for Dietary Studies (FNDDS) that translates the food as consumed into one or more ingredients (and gram amounts) or recipes. We then applied FNDDS version 2015-2016 food recipes (USDA 2018b) to process dietary recall data reported in NHANES 2013-2016 and FNDDS version 2013-2014

[43] Ibid

recipes (USDA 2016b) for foods that were only reported consumed in NHANES 2013-2014. The ingredients in the USDA recipe database were reviewed and the salmon codes that were identified from the USDA recipe database and used in the current analysis are summarized in figure 17. Only the proportion of foods corresponding to salmon was included in the analysis.

Figure 17 - Salmon ingredient codes included in analysis

Ingredient Code	Ingredient description
15077	Fish, salmon, chinook, smoked
15080	Fish, salmon, chum, canned, drained solids with bone
15083	Fish, salmon, pink, raw
15084	Fish, salmon, pink, canned, total can contents
15085	Fish, salmon, sockeye, raw
15087	Fish, salmon, sockeye, canned, drained solids

While there are no cooked salmon ingredient codes, the ingredient codes presented in figure 16 are reported consumed as is (i.e., raw, smoked, or canned without any additions) or as a component in foods by NHANES participants. Therefore, the salmon ingredient codes included in the analysis captured the reported consumption of salmon in the diet including cooked salmon. The NHANES food codes that contain salmon ingredients are provided below in Figure 18.

Figure 18 - NHANES salmon food codes included in assessment

Food Code	Food description
26100110	Fish, NS as to type, cooked, NS as to cooking method*
26100120	Fish, NS as to type, baked or broiled, made with oil*
26100122	Fish, NS as to type, baked or broiled, made with margarine*
26100123	Fish, NS as to type, baked or broiled, made without fat*
26100130	Fish, NS as to type, coated, baked or broiled, made with oil*
26100133	Fish, NS as to type, coated, baked or broiled, made without fat*
26100140	Fish, NS as to type, coated, fried, made with oil*
26100142	Fish, NS as to type, coated, fried, made with margarine*
26100160	Fish, NS as to type, steamed*
26100190	Fish, NS as to type, smoked
26137100	Salmon, raw
26137110	Salmon, cooked, NS as to cooking method*
26137120	Salmon, baked or broiled, made with oil*
26137121	Salmon, baked or broiled, made with butter*
26137122	Salmon, baked or broiled, made with margarine*
26137123	Salmon, baked or broiled, made without fat*
26137124	Salmon, baked or broiled, made with cooking spray*
26137130	Salmon, coated, baked or broiled, made with oil*
26137131	Salmon, coated, baked or broiled, made with butter*
26137133	Salmon, coated, baked or broiled, made without fat*

26137134	Salmon, coated, baked or broiled, made with cooking spray*
26137140	Salmon, coated, fried, made with oil*
26137143	Salmon, coated, fried, made without fat*
26137160	Salmon, steamed or poached*
26137170	Salmon, dried
26137180	Salmon, canned
26137190	Salmon, smoked
27250070	Salmon cake or patty*
27250080	Salmon loaf*
27450030	Salmon salad*
27450310	Lomi salmon*
58151200	Sushi roll, salmon*
58151420	Sushi, topped with salmon*

Flagging of statistically unreliable estimates

Per-user estimates of consumption that may be less statistically reliable due to inadequate sample sizes are flagged in the summary tables provided in the *Results* section of that document. The flagging of statistically unreliable estimates is based on guidance from NCHS (NCHS 1996). Specifically, estimates of mean consumption are flagged when based on a sample size of less than 30 times the variance inflation factor (VIF) and estimates of 90th percentiles of consumption are flagged when based on a sample size of less than 8 times the VIF and divided by 0.10 (i.e., 8 x VIF/0.1). VIF estimates of 1.98 and 2.41 were estimated by USDA for the NHANES periods 2013-2014 and 2015-2016, respectively (USDA 2016a, 2018a). Wildtype is not aware of a published VIF estimate for the combined NHANES 2013-2016.

In this analysis, a VIF of 2.41 from NHANES 2015-2016 (USDA 2018a) is assumed. Using the VIF of 2.41, estimated mean consumption is statistically unreliable if based on a sample size of less than 73 (30 x 2.41). Similarly, using a VIF of 2.41, the estimated 90th percentile consumption is statistically unreliable if based on a sample size of less than 193 (8 x 2.41/0.10).

Analysis

Using the WWEIA/NHANES consumption data, we estimated the 2-day average daily intake of salmon. For each subject with a complete 2-day dietary recall, salmon intake was derived by summing an individual's intake of salmon on day 1 and day 2 of the survey and dividing that sum by 2. If a survey participant consumed fish on only one of the survey days, that person's intake from day 1 was divided by 2 to obtain their 2-day average intake. Estimates were provided on a *per capita* and *per user* basis. Per capita estimates refer to the consumption based on the total population whereas *per user* estimates refer to those who reported consuming any fish or fish foods on either of the survey days.

The estimates based on 2-day average intakes do not necessarily represent long-term intakes, since they 1) may not capture infrequent consumers of occasionally eaten food, 2) assume that subjects who consumed such a food on both survey days actually consumed it

every day of the year, and 3) do not adjust for potential day-to-day variation in intake. A 2-day average typically overestimates long-term (chronic) daily intake and, therefore, does not necessarily represent long-term intakes.

All estimates of intake per person were generated using Exponent's Foods Analysis and Residue Evaluation Program (FARE® version 13.06) software. The statistically weighted values from the survey were used in the analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

Using the NHANES consumption data, the absolute and body weight (bw) adjusted EDI of salmon were derived for the total U.S. population and the following subpopulations: U.S. population two years of age and older (2+ years old), children 2-12 years old, adolescents 13-18 years old and adults 19+ years old. Intake estimates were provided at the mean and 90th percentile of intake and body weight-adjusted intakes were derived using each participant's measured body weight recorded in the NHANES examination component of the survey.

Results

The 2-day average intake estimate of salmon by the total U.S. population and subpopulations were calculated based on food consumption data collected in the WWEIA/NHANES 2013-2016. The *per capita* and *per user* mean and 90th percentile consumption estimates of salmon in grams per day (g/day) are provided in Figure 19. Body weight adjusted intakes (i.e., g/kg-bw/day) are provided in Figure 20.

Figure 19: Two-day average intake of salmon by the total US population (g/day); NHANES 2013-2016

			Per Capita		Per User	
Population	N-user [a]	% User	Mean	90th Percentile	Mean	90th Percentile
			---- g/day ----			
Total U.S.	697	4.9	3.0	0	60.2	109
U.S. 2+ y	687	5.0	3.1	0	60.5	109
Children 2-12 y	60[b]	1.6	0.7	0	39.8	81.2
Adolescents 13-18 y	63[b]	2.6	1.5	0	58.0	112
Adults 19+ y	564	6.0	3.7	0	61.7	109

[a] Un-weighted number of consumers; % user, *per capita*, and *per user* estimates were based on NHANES 2013-2016 and derived using the statistical weights provided by the National Center for Health Statistics (NCHS).
[b] Per user estimates at the mean and 90th percentile of intake is statistically unreliable due to inadequate user sample size.

Figure 20: Two-day average intake of salmon by the total US population (g/kg-bw/day); NHANES 2013-2016

			Per Capita		Per User	
Population	N-user[a]	% User	Mean	90th Percentile	Mean	90th Percentile
			---- g/kg-bw/day ----			
Total U.S.	697	4.9	0.04	0	0.84	1.54
U.S. 2+ y	687	5.0	0.04	0	0.83	1.54
Children 2-12 y	60[b]	1.6	0.02	0	1.43	2.56
Adolescents 13-18 y	63[b]	2.6	0.02	0	0.97	2.55
Adults 19+ y	564	6.0	0.05	0	0.80	1.48

[a] Un-weighted number of consumers; % user, *per capita*, and *per user* estimates were based on NHANES 2013-2016 and derived using the statistical weights provided by the National Center for Health Statistics (NCHS).
[b] Per user estimates at the mean and 90th percentile of intake is statistically unreliable due to inadequate user sample size.

References for intake estimates[44, 45, 46, 47, 48, 49, 50]

3.7 Safety assessment examples for select inputs

While the data in Figure 14 make it clear that the levels of exposure for inputs are *de minimis*, given that there are no published safety assessments available to the public for cell cultivated seafood, we are providing transparency on several inputs used in Wildtype's downstream processes that do not have significant existing precedents in food. The exposure levels and general safety discussion represented by these three inputs can generally be extended to all of the inputs summarized in Figure 14.

Fibroblast Growth Factor

Growing animal cells in a low/serum-free cell culture medium requires the addition of proteins and growth factors to support healthy cell proliferation. One such example is fibroblast growth factor-2 (FGF2), also known as basic fibroblast growth factor, bFGF, CASRN

[44] National Center for Health Statistics (NCHS). 1996. Analytic and Reporting Guidelines: The Third National Health and Nutrition Examination Survey, NHANES III (1988-94). Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention Accessed using this Link.
[45] National Center for Health Statistics (NCHS). 2016. National Health and Nutrition Examination Survey Data 2013-2014. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Accessed using this link.
[46] National Center for Health Statistics (NCHS). 2018. National Health and Nutrition Examination Survey Data 2015-2016. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Accessed using this link.
[47] U.S. Department of Agriculture, Agricultural Research Service. 2016a. Table 1 – Nutrient Intakes from Food and Beverages: Mean Amounts Consumed Per Individual, by Gender and Age, in the United States, 2013-2014. Accessed using this link..
[48] U.S. Department of Agriculture, Agricultural Research Service. 2016b. USDA Food and Nutrient Database for Dietary Studies 2013-2014. Food Surveys Research. Accessed using this link.
[49] U.S. Department of Agriculture, Agricultural Research Service. 2018a. Table 1 – Nutrient Intakes from Food and Beverages: Mean Amounts Consumed Per Individual, by Gender and Age, in the United States, 2015-2016. Accessed using this link.
[50] U.S. Department of Agriculture, Agricultural Research Service. 2018b. USDA Food and Nutrient Database for Dietary Studies 2015-2016. Food Surveys Research. Accessed using this link.

106096-93-9, a protein and ligand for multiple FGF receptors (FGFR), with essential roles in regulating cell survival, division, differentiation, and migration.[51]

A general search for information pertaining to the preclinical and clinical safety of FGF2 was conducted in the Google search engine as well as in databases that included the FDA-Approved Drugs database, https://www.clinicaltrials.gov/, and PubMed. With regard to safety, the oral route of exposure is the most relevant route to consider for this safety assessment of FGF2. Overall, no oral preclinical or clinical safety information was identified for FGF2, except for a one-month parenteral (i.e., non-oral) toxicity study of recombinant human bFGF conducted in dogs that reported a NOAEL > 480 mg/kg/day.[52] A study of enteral (oral) FGF2 efficacy as treatment for gastric ulcers is described further below, although toxicity was not a defined primary endpoint.[53]

Serum reference values for FGF2 were reported from a study conducted in 80 healthy blood donors.[54] The study authors determined that the reference values of FGF2 in men and women are <4.0 ng/l and <10.8 ng/l, respectively. Considering that the human blood volume is approximately 5 L and 55% of the blood volume consists of serum[55, 56], it can be estimated that <11 and <29.7 ng of FGF2 is contained in the serum of men and women, respectively.

Based on the mass-balance predicted concentration of FGF2 in Wildtype's cultivated salmon (1.0×10^{-15} ppm) and the 90th percentile intake of salmon (109 g/day), the highest estimated daily intake (EDI) is 1.09×10^{-10} ng/day (US population). This predicted exposure is infinitesimally small and trivial in comparison to the circulating level (<11 and <29.7 ng) in humans, and, hence is not of safety concern.

Pubmed and Google searches were performed to identify levels in muscle and other edible tissues of food producing animals, using terms such as cattle, cow, beef, chicken, bovine, pork, pig, fish, human food, etc. Serum level data when available are also summarized. As noted in Section 3.5, the peak concentration of FGF2 in rainbow trout has been quantified in the range of 10-100 ng/ml[57] and Wildtype's production processes never involve levels higher than these physiological concentrations.

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[51] Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, Gao G, Goldfarb M. Receptor specificity of the fibroblast growth factor family. J Biol Chem. 1996 Jun 21;271(25):15292-7. doi: 10.1074/jbc.271.25.15292. PMID: 8663044.

[52] Kim MY, Shin MK, Son JW, Kwak HI, Fang MZ, Bae MO, Kim JH, Cho MH, Kang KK, Kim WB, Ahn BO. One-month parenteral toxicity study of recombinant human basic fibroblast growth factor in dogs. Vet Hum Toxicol. 2000 Aug;42(4):234-5. PMID: 10928692.

[53] Hull MA, Knifton A, Filipowicz B, Brough JL, Vautier G, Hawkey CJ. Healing with basic fibroblast growth factor is associated with reduced indomethacin induced relapse in a human model of gastric ulceration. Gut. 1997 Feb;40(2):204-10. doi: 10.1136/gut.40.2.204. PMID: 9071932; PMCID: PMC1027049.

[54] Larsson A, Sköldenberg E, Ericson H. Serum and plasma levels of FGF-2 and VEGF in healthy blood donors. Angiogenesis. 2002;5(1-2):107-10. doi: 10.1023/a:1021588227705. PMID: 12549867.

[55] Feher, J. (2012). 5.13 - Regulation of Arterial Pressure. In J. B. T.-Q. H. P. Feher (ed.), (538–548). Boston: Academic Press.

[56] Mathew J, Sankar P, Varacallo M. Physiology, Blood Plasma. 2021 Apr 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 30285399.

[57] Jun-ichiro Hata, Jiro Takeo, Shinya Yamashita, Heparin Essential for Tissue Culture with Fish Fibroblast Growth Factor 2, Fisheries science, 1998, Volume 64, Issue 2, Pages 216-219, Released June 30, 2008, Print ISSN 0919-9268, https://doi.org/10.2331/fishsci.64.216

Although the concentration of FGF2 (and all other growth factors) never exceeds the physiological ranges encountered in conventional seafood during Wildtype's production processes, the company has nevertheless assessed the potential effects of consuming products made with protein growth factors. To begin, the stability of growth factors degrades quickly with temperature fluctuations, with both heating[58] and freezing[59]; for this reason, Wildtype's lethal thermal processing stage and standard cold storage serve to denature and inactivate such growth factors. In the case of FGF2, the denaturation / inactivation temperature has been quantified as the T_m (denaturation point); at physiological pH values, the characterized T_m range for FGF2 spans 59˚C[60] to 63˚C[61]. In studying the rate of FGF2 degradation at 65˚C, Dvorak et al. were not able to obtain half-life measurements "because the proteins were denatured immediately at the beginning of the measurement."[62] Wildtype's 2-hour lethal processing stage at 65˚C therefore serves to effectively denature and inactivate FGF2 prior to packaging.

An additional mechanism of further growth factor degradation is the presence of proteases (protein-degrading enzymes) created by cells in culture. These proteases are naturally synthesized by cells, and are abundant in both cell culture[63] and conventional seafood[64]; they degrade proteins over time, further ensuring the inactivation of functional growth factors in cultivated seafood products. Wildtype's production process does not involve the addition of protease inhibitors, thus permitting ongoing protein degradation by endogenous proteases found in cell culture.

Although the above conditions (starting FGF2 concentrations below conventional seafood, wash steps for further dilution, thermal denaturation, and protease-mediated proteolysis) make it exceedingly unlikely that any functional growth factor is present in the final product, the potential for growth factor ingestion merits consideration. Given the temperature and pH sensitivities of FGF2, it is assumed that any remaining FGF2 behaves like other proteins that are rapidly proteolyzed and neutralized during the early stages of digestion as they are exposed to proteases such as pepsin and trypsin in the acidic environment of the proximal gastrointestinal tract.[65]

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[58] Wang, Y. J. et al. Characterization, stability, and formulations of basic fibroblast growth factor. Adv Exp Med Biol 9, 141–80 (1996).

[59] Benington, L. R., Rajan, G., Locher, C. & Lim, L. Y. Stabilisation of Recombinant Human Basic Fibroblast Growth Factor (FGF-2) against Stressors Encountered in Medicinal Product Processing and Evaluation. Pharm 13, 1762 (2021).

[60] VEMURI, S. et al. The Stability of bFGF Against Thermal Denaturation. J Pharm Pharmacol 46, 481–486 (1994).

[61] Wang, Y. J. et al. Characterization, stability, and formulations of basic fibroblast growth factor. Adv Exp Med Biol 9, 141–80 (1996).

[62] Dvorak, P. et al. Computer-assisted engineering of hyperstable fibroblast growth factor 2. Biotechnol Bioeng 115, 850–862 (2018).

[63] Clincke MF, Guedon E, Yen FT, Ogier V, Goergen JL. Characterization of metalloprotease and serine protease activities in batch CHO cell cultures: control of human recombinant IFN-γ proteolysis by addition of iron citrate. BMC Proc. 2011 Nov 22;5 Suppl 8(Suppl 8):P115. doi: 10.1186/1753-6561-5-S8-P115. PMID: 22373384; PMCID: PMC3285009.

[64] Haard, NF and Simpson, BK. Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality (Food and Science Book 97). CRC Press (2000). ISBN: 0367398885

[65] Hasler WL, Owyang C. Approach to the Patient with Gastrointestinal Disease. In: Jameson J, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. eds. *Harrison's Principles of Internal Medicine, 20e*. McGraw Hill; 2018.

Finally, it is worth noting that protein growth factors, at concentrations that are orders of magnitude greater than those considered here, have been investigated as therapeutic agents for conditions ranging from short bowel syndrome[66] to inflammatory bowel disease[67]. In the case of FGF2, one study compared the ingestion of an acid-resistant variant of this growth factor (100µg twice daily, or 1 x 10^{15} times greater than the maximal theoretical daily intake of FGF2 in Wildtype salmon) to placebo and standard pharmacological treatment in the context of relapsed gastric ulceration. There were no reported side effects, pathological sequelae, or irregularities noted with endoscopy and biopsy in any of the 3 treatment conditions.[68]

The safety of ingested growth factors such as FGF2 in conventional seafood and in the therapeutic examples above (both at higher concentrations than those found in Wildtype's products) overarches the exceedingly unlikely probability that physiologically relevant concentrations of active growth factors are present in Wildtype's final products.

Taurine

Taurine is GRAS, through scientific procedures, for use as an ingredient in non-carbonated, flavored, water-based beverages at a level of 0.0045% (GRN586). The GRAS use of taurine was estimated to result in a 90th percentile intake of 29.3 mg/day (0.45 mg/kg bw/day). FDA issued a no question letter (GRN 586, December 9, 2015).

A comprehensive search of the literature bearing on the safety of taurine was conducted in January 2022. Databases that were searched included PubMed, PubChem, ScienceDirect, BioMed Central, Google and Google Scholar, the U.S. EPA IRIS/HPVIS, the National Toxicology Program (NTP), OECD, Inchem, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), the European Commission Scientific Committee on Food, and the U.S. Food and Drug Administration (FDA).

The findings from the literature search/review indicated that EFSA completed a review of the safety of ingestion of taurine as an ingredient in energy drink beverages. This assessment, which is publicly available, includes a review of published and unpublished toxicity testing, and a review of clinical studies. This assessment and the data summarized therein along with other publicly available safety data are used as the basis of the following safety summary.

[66] McMellen, M. E., Wakeman, D., Longshore, S. W., McDuffie, L. A. & Warner, B. W. Growth factors: possible roles for clinical management of the short bowel syndrome. Semin Pediatr Surg 19, 35–43 (2010).

[67] Triantafillidis, J. K., Tzouvala, M. & Triantafyllidi, E. Enteral Nutrition Supplemented with Transforming Growth Factor-β, Colostrum, Probiotics, and Other Nutritional Compounds in the Treatment of Patients with Inflammatory Bowel Disease. Nutrients 12, 1048 (2020).

[68] Hull MA, Knifton A, Filipowicz B, Brough JL, Vautier G, Hawkey CJ. Healing with basic fibroblast growth factor is associated with reduced indomethacin induced relapse in a human model of gastric ulceration. Gut. 1997 Feb;40(2):204-10. doi: 10.1136/gut.40.2.204. PMID: 9071932; PMCID: PMC1027049.

For taurine, the highest daily dose used in a randomized, controlled trial was up to 10g (Durelli et al., 1983, as cited in Shao and Hathcock, 2008).[69] However, the study had several limitations, including small sample size and lack of clinically relevant safety outcome measures.

Chauncey et al. (2003, as cited in Shao and Hathcock, 2008) administered 3g/day to type 2 diabetic patients (n = 22) for 4 months. There was a significant 33% increase in serum taurine, no effect on glycosylated hemoglobin (HBA1C) or fasting glucose levels, and no adverse effects were reported. Shao and Hathcock concluded that the small sample size and modest duration of the study argued against its use for identification of an OSL.

Additional studies in T2DM patients conducted by Esmaelli et al. (2020)[70] and Meleki et al. (2020)[71] of 3 g/day of taurine showed reduced fasting blood sugar, insulin, HOMA-IR, total cholesterol, low-density lipoprotein cholesterol, improved metabolic profiles, and pentosidine and methylglyoxal levels in the patients with type 2 diabetes mellitus. No safety data were reported in these studies. Additionally, the authors noted that these studies were limited due to short durations and small sample sizes.

Zhang et al. conducted two studies in which healthy adults were administered 3g taurine/day for 12 days (Zhang et al., 2004a, as cited in Shao and Hathcock, 2008) and 7 weeks (Zhang et al., 2004b, as cited in Shao and Hathcock, 2008), respectively. In the shorter of the two trials (n = 13) there was a significant 8-fold increase in urinary taurine and no adverse effects were reported. In the longer of the two (n = 15), there was a significant decrease in serum triglycerides and no change in HDL cholesterol or fasting glucose. No adverse effects were reported. Shao and Hathcock concluded that despite the small sample size and modest duration, the lack of significant adverse effects observed in this trial involving healthy adults and in other trials using doses equal to, above and below 3 g/day, supports a risk assessment based on the observed safe level (OSL).

Several other trials have been conducted at doses ranging from 0.4 to 1.5g/day for up to 1 year. They were all relatively small studies in which there were no changes in biochemical measurements and/or no adverse effects observed (Colombo et al., 1996; Sirdah et al., 2002; Brons et al., 2004; Spohr et al., 2005; Cangemi, 2007, as cited in Shao and Hathcock, 2008).

Based on the collection of clinical studies, Shao and Hathcock (2008) considered the NOAEL and LOAEL to be >10 g taurine/day and the OSL and upper intake level (UL) to be 3g/day. The OSL method was published by the Council for Responsible Nutrition (CRN) Vitamin and Mineral Safety, 2nd edition (Hathcock 2004) and contains the basic features of the Food and Nutrition Board's usual safe upper intake level (UL) method. If no adverse effects in humans

[69] Shao, A. and Hathcock, J.N. 2008. Risk assessment for the amino acids taurine, L-glutamine, and L-arginine. Reg. Toxicol. Pharm. 50: 376-399.

[70] Esmaeili, F., Maleki, V., Kheirouri, S., & Alizadeh, M. (2021). The Effects of Taurine Supplementation on Metabolic Profiles, Pentosidine, Soluble Receptor of Advanced Glycation End Products and Methylglyoxal in Adults With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial. Canadian journal of diabetes, 45(1), 39–46.

[71] Maleki, V., Alizadeh, M., Esmaeili, F., & Mahdavi, R. (2020). The effects of taurine supplementation on glycemic control and serum lipid profile in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Amino acids, 52(6-7), 905–914.

can be established, one identifies the highest intake level with sufficient evidence of safety as the OSL (equivalent to the highest observed intake (HOI) established by FAO/WHO). The OSL method is an acceptable substitute for the traditional ADI/EDI approach using animal data and the application of safety factors to identified NOAELs.

In 2012, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered EFSA's 2009 review of the available data on taurine consumption in infants, children, and adults along with the EFSA (2009) conclusion that oral daily ingestion of taurine in the 3-6g dose range for periods up to one year did not cause any adverse health effects. Based on EFSA's 2009 evaluation of taurine, FEEDAP (2012) determined an OSL in humans to be 6g/day.

Based on the mass-balance predicted concentration of taurine in cell-cultivated salmon (2.2 x 10^{-12} ppm) and the 90th percentile intake of salmon (109g/day), the highest estimated daily intake (EDI) in mg/day is 2.40 x 10^{-10} (US population). This predicted exposure is infinitesimally small and a trivial fraction of the existing dietary exposure of 29.3mg/day (GRN 586), and well below the OSL in the range of 3-6g/day. Therefore, it is not of safety concern.

Nicotinamide adenine dinucleotide

NAD is an essential coenzyme that plays a crucial role in the electron-transfer reactions integral to the basic metabolic processes responsible for energy production in all forms of life (Linus Pauling Institute, 2018). Over 400 enzymes require NAD or its counterpart, nicotinamide dinucleotide phosphate (NADP), for these reactions. NAD in its oxidized state, NAD+, is used in catabolic pathways where macromolecules (e.g., carbohydrates, lipids, proteins) are broken down via oxidation. NAD accepts the electrons during the oxidation reaction, thus becoming reduced (NADH) (Linus Pauling Institute, 2018).

Most dietary niacin is in the form of nicotinic acid and nicotinamide, but some foods contain small amounts of NAD and NADP, such as milk (Ummarino et al., 2017; Trammell et al., 2016a). In ovine milk, most of total niacin seems to be represented by NAD, but in bovine and human milks, NR and NMN may account for a significant amount of the total niacin content (see Table 1). Overall, existing exposure to NAD is established mainly via dietary exposure to its precursors: niacin, NR and tryptophan.

History of Use: The average dietary exposure to niacin among US adults is in the range of 21.3 – 36.3mg/day, providing an NAD equivalent exposure in the range of 116 – 197mg/day. Based on EU intake data, the 95th percentile niacin intake in adults can be as high as 78.2mg/day or an equivalent of 425mg NAD/day. The proposed use of NAD as a dietary supplement delivering a daily dose of 300mg/day is higher than the typical exposure in the US diet, but likely below upper percentile intake. Also, in the US, the use of NR (Niagen) as a dietary supplement at a daily dose of 180mg/day is providing an equivalent of 411mg NAD/day. Based on the mass balance predicted concentration of NAD n cell-based salmon (3.6 x 10^{-12} ppm) and the 90th percentile intake of salmon (109g/day), the highest estimated daily intake

(EDI) in mg/day is 3.92 x 10^{-13} (US population). This predicted exposure is infinitesimally small and a trivial fraction of the existing dietary exposure to NAD, and therefore, is not of safety concern.

Comparison with NOAEL for NADH: Based on the mass balance predicted concentration in cell-cultivated salmon (3.6 x 10^{-12} ppm) and the 90th percentile intake of salmon of 2.56g/kg bw/day (children 2-12 years old), the highest EDI is 9.22 x 10^{-15} mg/kg bw/day. This predicted worst case EDI is well below the lowest NADH NOAEL (7.9mg/kg bw/day), with a very large margin of exposure (MOE) of 8.57 x 10^{14}, and thus, not of safety concern.

3.8 Guiding principles from FDA's preventive controls and seafood HACCP requirements

FDA's Food Safety Modernization Act (FSMA) is aimed at identifying potential safety hazards *a priori* and implementing preventive controls rather than reacting to issues as they occur.[72] Accordingly, Wildtype has engaged in an iterative process of hazard identification and implementation of preventive controls over the last two and a half years, not only to ensure that safety standards for its seafood products are no less stringent than for conventional seafood, but also to maintain consistency in its production practices. For example, the introduction of adventitious agents during the cell proliferation stage not only creates a potential food safety risk, but these microorganisms would likely outcompete cells and overtake the culture, leading to a costly loss of product.

Guiding principle #1: implement good manufacturing practices

As elaborated in 21 CFR 117 subpart B, good manufacturing practice (GMP) requires that personnel maintain cleanliness during food processing by wearing outer garments, washing hands frequently, wearing gloves when required, and separating personal items from food processing areas. Limiting exposure to disease, promoting education and training, and ensuring adequate supervision are also critical components of GMP-compliant production. The nature of cell culture work not only requires the same principles of cleanliness, education, training, and supervision, but often demands an even higher standard due to the potential of microorganisms to contaminate cell culture. As such, Wildtype personnel are already operating at a level that in many ways exceeds GMP standards within 21 CFR 117 subpart B.

With respect to facility and grounds standards, cell culture work demands a similarly high degree of cleanliness and pest control as described in 21 CFR 117.20. Wildtype has upgraded its current production facility substantially to include 24/7 climate control, sealed doors and windows, and other precautionary measures to improve food safety.

Guiding principle #2: limit potential for allergen cross-contact

[72] Seafood HACCP and the FDA Food Safety Modernization Act: Guidance for Industry, US Department of Health and Human Services, FDA, Center for Food Safety and Applied Nutrition, August 2017.

All of Wildtype's current production centers on only one species: coho salmon *(Oncorhynchus kisutch).* As such, the potential for allergen cross-contact during production is negligible.

The majority of Wildtype's current research and development activities concern the production of various salmon *genera*. As discussed in the cell banking and cryopreservation section above, vials containing different species of salmon cells are kept apart in liquid nitrogen storage to limit the potential for cross-species contamination. Wildtype will implement a label review program that will verify and document that the proper allergen label is on all production lots prior to release into commerce.

Guiding principle #3: hazard analysis and risk-based preventive controls

As discussed in 21 CFR 123, seafood processors are required to maintain a Hazard Analysis and Critical Control Point (HACCP) plan. A revised food safety plan was completed in July 2021 in partnership with Texas Tech University. This process identified a number of risk mitigation steps that the company is implementing. As discussed above, many of these risk mitigation measures are required for standard operations of a cell culture facility. Additionally, as Wildtype's production processes (and associated material inputs) have evolved, new risk mitigation strategies have been developed and implemented at the company.

A number of potential hazards specific to seafood products are listed in 21 CFR 123.6. These hazards are listed below along with the applicability within the context of Wildtype's production technology.

- Natural toxins: limited exposure; see Source cell procurement and health assessment section above
- Microbiological contamination: relevant hazard; mitigated by aseptic technique and periodic microbiological testing
- Chemical contamination: limited exposure due to controlled production environment
- Pesticides: not relevant
- Decomposition of scombroid toxin-forming species: not relevant
- Parasites: not relevant
- Unapproved use of direct or indirect food or color additives: our materials hazard analyses support a conclusion that our process does not result in the unapproved use of direct or indirect food or color additives

Wildtype considers the finished product manufactured at its facility to be ready-to-eat (RTE). Although cleaning and sanitation procedures have not been finalized, the area where product is harvested and packaged will likely require clean-out-of-place procedures; therefore *L. monocytogenes* will be considered the most likely pathogen of concern. During the buildout of the facility and creation of the production line, Wildtype will follow FDA's guidance for control of *L. monocytogenes* in RTE foods. More details on Wildtype's environmental monitoring program to include environmental pathogens such as L. monocytogenes and Salmonella serovars may be found in section 3.10 below.

Guiding principle #4: record keeping

Wildtype is implementing both a documentation control program and a production batch record keeping system. The documentation control system identifies, organizes, and tracks revisions to all documents pertaining to food safety and the company's quality system. It provides standard documentation including a reference number, document title, current version or issue number, issue date, and withdrawal status.

The company's production batch record system tracks the name of material inputs, the supplier, lot number, material safety data sheet, and supplier certificate of analysis, when provided. This system will be used to track every input used during commercial production in order to facilitate and track corrective actions should they become necessary. The production batch records will be reviewed by Quality Assurance to verify all quality and safety standards were met prior to release of the product into commerce.

Guiding principle #5: supply-chain program

Wildtype is implementing a supplier approval program which includes the following steps:

1. Hazard assessment
2. Review of supplier qualifications
3. Incoming material inspections
4. Monitoring of supplier performance including annual audits
5. Notice of supplier non-conformance and corrective actions, as required
6. Supplier approval checklist
7. Record keeping

Supplier qualifications and approvals are based on the hazard assessment of the materials in question with respect to potential chemical, biological, or physical hazards. Routine visual and physical inspection of incoming materials is currently part of Wildtype's standard operating procedures. Wildtype plans to use only qualified suppliers, and will provide a corrective action based on the hazard assessment if an unqualified supplier must be used.

3.9 Current and planned testing and monitoring

A number of routine tests are currently used during Wildtype's product development activities. Some assays are carried out via real-time controls such as pH monitoring, and others are conducted via periodic sampling and send-out analyses. These include:

1. Microorganism monitoring and testing: bacteria, yeast, and fungi
2. *Mycoplasma* testing
3. Nutritional comparability testing (e.g., protein, fat, vitamins)
4. Periodic common contaminant testing

As part of its quality control processes, Wildtype plans to implement routine sampling and testing of these assays for initial batches of Wildtype seafood products, and to screen finished products for the material inputs discussed in earlier sections of this analysis.

3.10 Environmental Monitoring Program

Wildtype will also implement an environmental monitoring program to evaluate potential niches of both pathogens of concern and indicator organisms within the facility.

The first step in establishing Wildtype's environmental monitoring program was to identify hygienic zoning. Referencing Figure 1, all of Wildtype's "upstream" production steps and "downstream" steps through "cell harvest" involve handing cells either under tightly controlled aseptic technique as described above or in closed bioreactor systems. These areas are classified as low- or medium-care areas. Downstream steps including scaffold creation and scaffold seeding are currently exposed to the environment and take place in a dedicated high-care area. These areas include upgraded HVAC systems (positive pressure and HEPA filtration). Additionally, per GMPs, staff entering these areas require additional training. Access to these high-care areas is controlled with badging requirements and require additional boot cleansing and gowning steps.

In addition to hygienic zoning, a baseline environmental study will be completed prior to moving our facility into production. These studies will be conducted in accordance with standard practices in food safety.[73] Levels of indicator organisms will be established in order to specify safety thresholds.

Wildtype will conduct weekly testing of product and non-product contact surfaces throughout the facility. Monitoring for these purposes will be validated by 3rd party testing services. In the case of microorganism monitoring, gold standard culture testing of sampled environments will be performed. For example, Tryptone Soya Bean (TSB) agar bacteriological culture places can be used for this purpose. Open TSB agar plates are placed on work surfaces at strategically placed locations in the lab to assess the airborne microbial loads. While TSB is a non-selective culture media and is best used as a baseline to establish baseline microorganism levels, it will be supplemented with contact surface testing for the pathogens of interest, *L. monocytogenes* and *Salmonella* serovars. This testing strategy will be scaled up as the company's facility size increases, and encompasses a comprehensive assessment of all environmental locations with the potential to represent a pathogenic nidus. Trending these data will allow Wildtype to monitor its facility to ensure potential contamination during and after harvest is minimized.

If a sample shows indicator organisms above the established thresholds, additional sanitation will be conducted. After the intervention, increased sampling will be conducted on

[73] American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods. 4th Edition. 2001. Chapter 3.

the identified areas to confirm that the new sanitation protocols have reduced indicator organisms to the acceptable level.

3.11 Allergens

Because cultivated salmon contains cells derived from Pacific salmon species, the company expects those with allergies to conventional salmon will have a similar response to its salmon products. As such, the company intends to provide all required allergen notices on its packaging, consistent with standard seafood labeling guidelines.

A screen for expected allergens, including those found in scaffolds, has been conducted to determine the presence of these allergens in its products. In addition to the fish-specific allergens described above, soy protein was identified as a sole potential allergen; per standard practice, required allergen labeling will be provided on packaging.

3.12 Food contact surfaces

There are a number of food contact surfaces used in the production of Wildtype's products. As a general principle, any food contact surface in use by Wildtype during its production processes is comprised of substances that are permitted for their intended use in contact with food. The list below summarizes the food contact surfaces in use during the various process steps:

- Source cell procurement, cell line establishment, and characterization: plastic and glass vials, cell culture dishes, and flasks
- Cell banking and cryopreservation: same as above with the addition of plastic 1mL cell storage vials that are submerged in liquid nitrogen for long-term storage
- Cell proliferation: stainless steel at or exceeding grades of 316 in order to minimize corrosion hazards
- Cell harvest: cell collection takes place in stainless-steel filtration units; cells are stored in standard cell culture flasks and dishes and refrigerated or frozen until they are seeded in scaffolds
- Scaffold production and cell seeding: stainless steel mixing units, pans, and sheets as well as molds fabricated from nylon 12, a food contact surface approved for wide use[74]
- Packaging: standard food packaging currently in use for seafood products

All equipment purchased will be evaluated on its ability to be cleaned and sanitized. Wildtype will avoid equipment with hard to clean areas such as rough seam welds or hard to reach junctures. All SSOPs will be validated prior to being used in production. Sanitation crews will be trained to implement SSOPs and retrained annually. Wildtype management will periodically evaluate sanitation crews on adherence to SSOPs.

[74] 21 CFR 177.1500

3.13 Shelf life

Formal shelf-life testing is underway. We have established relationships with several universities that are able to conduct these studies prior to commercialization.

4. Conclusion

The safety assessment contained in this document accounts for all inputs and potential hazards involved with the production of cultivated salmon. It also demonstrates that the primary components, salmon cells, are genetically indistinguishable from those of conventional salmon, and that other inputs are not present in the final product at meaningful levels. For these reasons, we consider the output of this cell production process to be salmon. Substances used in scaffolds are widely used in food production today in a manner consistent with Wildtype's use, and are used consistent with applicable regulatory requirements. We therefore conclude that Wildtype's products meet applicable safety requirements and may be lawfully introduced into interstate commerce.

Received: 10 November 2022
Responded: 17 January 2023

Overview

This document responds to the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 10 November, 2022. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests

Source Cell Procurement and Health Assessment

1: Identity - Information Requested

On page 5 of the disclosable safety narrative, you state "Wildtype has developed its salmon cell lines from egg, alevin, and fry stages of development", and provide a health certificate from a Canadian institute, The BC Centre for Aquatic Health Sciences Society. On page 7 of the disclosable safety narrative, you mention isolation from fish tissue (i.e., muscle biopsy). However, the source cell species is not specified in the disclosable safety narrative, nor is the source of the salmon (i.e., from aquaculture or wild caught). Pages 15, 16, and 53 of the disclosable safety narrative refer to the harvested cell material as "Pacific salmon" or being derived from Pacific salmon; however, Pacific salmon has several species. On page 9 of the disclosable safety narrative, you state that "… the DNA in various Wildtype cell lines is unequivocally Coho salmon DNA".

Please specify, for addition to the disclosable safety narrative:

- the source of the cell species;
- whether the source cell species is from aquaculture or if it is wild caught. If the source cell species is from both, please specify whether there are any differences in your hazard analysis and preventative controls;
- whether the salmon cell line used to derive the harvested cellular material that is the subject of CCC 000005 was developed using different cell species from sources in the United States or Canada; and
- information about the myoblasts and fibroblasts isolated from the salmon muscle. This information should be provided in detail to present any biological hazards identified, and the controls established to mitigate any potential microbial contamination from the salmon muscle. Similarly, please provide information regarding biological hazards identified, and the controls established to mitigate any potential microbial contamination from the salmon eggs.

Significance

It is important to distinguish if the source cell species is from aquaculture or if it is wild caught, as the biological hazard may differ depending on the source. For example, Anisakis simplex larvae may occur in a variety of marine fish including wild salmon from different regions, as well as Coho salmon from

Japan[1]; however, A. simplex larvae are generally not found in farmed fish as the parasite originates from the live food consumed by wild fish.

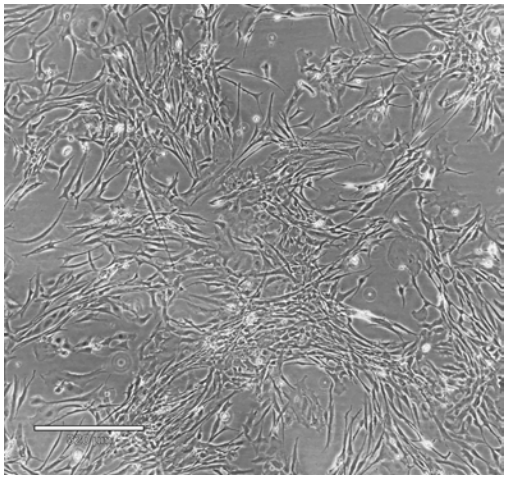
Wildtype Response: The salmon cell line described in CCC 000005 was derived solely from Coho salmon fry originating from a Washington state hatchery.

During cell line establishment, muscle and connective tissue (containing myoblasts and fibroblasts, respectively) are removed from the donor fish, minced, and treated with sterilizing agents such as hydrogen peroxide in order to neutralize potential adventitious agents that may have been conveyed by the donor fish from its environment, such as those described on pages 6-8 of CCC 000005.

Potential biological hazards relevant to cells isolated from both eggs and juvenile fish originating from hatcheries include those transmitted by human handling, environmental hazards, and transmission from the donor fish into cell culture such as *Listeria monocytogenes, Salmonella spp., Staphylococcus aureus,* and *E. coli*.

Each of these potential biological risks are mitigated by the same set of controls employed by Wildtype personnel. These include aseptic and Good Manufacturing Practices (GMP) techniques such as the use of laminar flow hoods, gloves, and gowning techniques. Sterile rinsing of the donor fish tissue using hydrogen peroxide eliminates a significant amount of the aforementioned potential pathogens. As described in CCC 000005, antibiotics are used for the first several passages in order to further mitigate the growth of adventitious agents in culture. Additionally, in these early stages, cell cultures are carefully monitored for contamination via microscope and changes in pH. In the event of a microbial contamination, salmon cells would be overtaken and the resulting contamination would be easily detectable via microscope. A representative micrograph of contamination-free cells in the early stage of culture is shown in figure 1 below.

Figure 1 – Early-stage culture of coho Pacific salmon cells (scale bar = 520µm)



[1] Roberts, T. A., et al., 2005. Fish and Fish Products, p. 174–249. In International Commission on Microbiological Specifications for Foods, Microorganisms in Foods 6. Microbial ecology of food commodities. Kluwer Academic/Plenum Publishers, New York. https://doi.org/10.1007/0-387-28801-5

Cultures displaying the presence of adventitious agents are terminated. A terminal thermal step at the end of Wildtype's production process (see further discussion in response to question 15 below) further mitigates the risk of any potential contamination, and routine lot testing for the presence of potential pathogens helps to ensure that controls employed earlier in the production process, including at the cell line isolation step, are effective.

Cell Line Establishment

2: Adventitious Agent Hazard Assessment - Information Requested

On page 6 of the disclosable safety narrative, you state "Clostridium perfringens, Campylobacter jejuni, Yersinia enterocolitica, E. coli, Shigella spp, and Salmonella spp when present in fish are generally from sewage pollution or terrestrial run-off. Salmonella spp risks in food production facilities stem largely from human handling and would be relevant to Wildtype's production technology". Chapter 4 of the Bacteriological Analytical Manual[2] states, "Detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary condition in the food-processing environment. Fecal coliforms remain the standard indicator of choice for shellfish and shellfish harvest waters; and E. coli is used to indicate recent fecal contamination or unsanitary processing". Further, in 1998 soy sauce marinated salmon roe caused a major outbreak of E. coli O157:H7 due to human handling during processing.[1,3] In light of this, and the observation about the utility of coliforms and E. coli as an indicator of sanitary conditions (including human handling) or fecal contamination, please describe, for addition to the disclosable safety narrative, some additional discussion about whether and why E. coli and coliforms are or are not considered relevant in your particular production process.

Significance

This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: The presence of *E. coli* and coliforms in Wildtype's production process can indicate the presence of pathogenic sub-species of *E. coli* or other pathogens as discussed in CCC 000005. As such, we have included *E. coli* and coliform panels in baseline and ongoing finished product testing as described in Figure 5 below.

The risk of pathogenic *E. coli* contamination at the cell isolation stage is mitigated by the use of aseptic technique and careful monitoring via visual inspection or rapid pH changes, as pathogenic *E. coli* would rapidly outcompete salmon cells in a cell culture environment. As discussed in CCC 000005, cells are passaged upwards of 50 times while establishing a cell line (cell cultures displaying any form of

[2] Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. Chapter 4. Accessible at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria

[3] Infectious Agents Surveillance Report (IARS) (1998). 19(10). [In Japanese]. Accessible at: https://idsc.niid.go.jp/iasr/19/224/inx224.html

contamination are terminated) so the risk of pathogenic *E. coli* conveyed via the donor animal and its environment is insignificant.

Pathogenic *E. coli* may also enter Wildtype's production environment and its products via human handling. Mitigating controls against *E. coli* include GMP precautions such as donning of plant clothes (e.g., dedicated shoes/shoe covers, masks, coats, hairnets, and eye protection). Finally, a terminal lethal step and initial testing of production lots for *E. coli* mitigates the risk of contamination.

3: Adventitious Agent Hazard Assessment - Information Requested

On page 6 of the disclosable safety narrative, you state "... Salmonella spp risks in food production facilities stem largely from human handling and would be relevant to Wildtype's production technology". On page 7, you state, "Viruses of human concern (Noroviruses and Hepatitis A) are predominantly found in shellfish and arise from contaminated water or human handling" but conclude that they are not a concern in the production process: "These hazards are not relevant to Wildtype's production system for several reasons. First, municipal water is used as a starting source, which is subject to EPA-regulated viral decontamination strategies and confers an exceedingly low initial risk of these viral contaminations. Second, direct human contact with water is not part of Wildtype's production methodologies, including all relevant processes of sterilization". The statement you provide on page 6 regarding the relationship between hazards from Salmonella serovars and human handling is not restricted to contamination of water. Please describe for addition to the disclosable safety narrative, whether there are potential opportunities for contamination with these viruses resulting from human handling of the cells or other substances used in the cell culture process, and if so, whether this hazard represents a meaningful food safety risk.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Noroviruses and Hepatitis A may be transmitted to food products via human handling.[4] Given the aseptic nature of cell culture (e.g., sterile technique and the use of laminar flow hoods), human transmission to fish cells is unlikely. In later stages of processing, however, potential contamination is possible during the scaffold creation, cell seeding, and final processing steps.

Mitigating controls for noroviruses and Hepatitis A include aseptic technique and hygienic zoning with additional gowning requirements in areas where higher-risk processing steps occur. All steps from cell culture to final processing also take place within a Current Good Manufacturing Practice (cGMP)

[4] O'Shea H, Blacklaws BA, Collins PJ, McKillen J, Fitzgerald R. Viruses Associated With Foodborne Infections. Reference Module in Life Sciences. 2019:B978-0-12-809633-8.90273-5. doi: 10.1016/B978-0-12-809633-8.90273-5. Epub 2019 May 21. PMCID: PMC7157469.

compliant facility to further mitigate the risk of human viral transmission. A thermal inactivation step[5,6] serves as an additional safeguard against viral contamination, although the emergence of heat-resistant strains of norovirus have recently been reported.[7] For this reason, norovirus and Hepatitis A virus testing of initial production batches are part of Wildtype's finished product testing plan (detailed in the response to question 15); test results of three non-consecutive batches of finished product is shown in Figure 6 below, demonstrating the absence of all pathogens.

Cell Bank Establishment

4: Microbial and Viral Testing - Information Requested

On page 11 of the disclosable safety narrative, you state "Lots are periodically tested for bacterial (aerobic and anaerobic microorganisms such as Staphylococcus spp. and Fusobacterium, respectively), as well as fungal contamination (such as Aspergillus) that may be introduced by cell culture operators at this stage. This testing is completed by third-party testing agencies. These tests are always conducted prior to submitting any vials to Wildtype's master cell bank". Please clarify, for addition to the disclosable safety narrative, the discrepancy between your statement that "Lots are periodically tested for" and "These tests are always conducted".

Further, for addition to the disclosable safety narrative, please clarify the relationship of these statements with your discussion of your adventitious agent hazard assessment on pages 6 and 7, and the "Hazard Analysis and Preventive Controls" table presented in Figure 12.

Significance

This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: We have updated our cell banking procedures since submitting CCC 000005. At least one vial of every lot that is submitted to Wildtype's master cell bank is tested by a third-party accredited laboratory for the aforementioned potential bacterial and fungal contaminants. Third-party testing for these potential adventitious agents is also completed during any master cell bank maintenance, which includes one thaw test every six months to ensure that cell viability, doubling times, and cell morphology are consistent with production specifications.

Master cell banking procedures are focused on the potential hazards present at this stage of the production process, which are limited to the bacterial and fungal contaminants listed above. The other hazards listed on pages 6 and 7 or Table 12 of the disclosable safety narrative in CCC 000005 are not significant risks due to the use of aseptic technique employed during cell culture and the master cell

[5] CDC guidance on heating requirements in food to inactivate noroviruses may be found here
[6] CDC guidance on heating requirements in food to inactivate hepatitis A may be found here
[7] Tan MTH, Xue L, Wang D, Eshaghi Gorji M, Li Y, Gong Z, Li D. The globally re-emerging norovirus GII.2 manifests higher heat resistance than norovirus GII.4 and Tulane virus. J Appl Microbiol. 2022 Mar;132(3):2441-2449. doi: 10.1111/jam.15379. Epub 2021 Nov 30. PMID: 34821445.

banking process. A terminal lethal step is employed to mitigate potential contamination. Testing lots of finished product during the early stages of commercialization until a baseline is established for the effectiveness of the other described mitigations will provide additional safeguards against the introduction of other potential adventitious agents.

Substances Used During Cell Culture

5: Safety Assessment - Information Requested

On page 31 of the disclosable safety narrative, you describe your general safety assessment process for the substances used in the culture medium, including a comparison of estimated daily intake values to safety thresholds you have identified ("e.g., scientific literature or the Threshold for Toxicological Concern (TTC)"). Where scientific literature, such as a published "no observed effect level" from a toxicological study for the substance or a related substance was used, please provide this information, including the complete citation and an estimate of the margin of exposure. If some other point of reference, such as existing authorized uses or levels commonly found in food was used, please provide this point of reference and estimated margin of exposure. As we have previously stated, reference levels such as a TTC are only appropriate for certain types of substances and not others (e.g., proteins) based on the process that was used to develop these reference levels.

Significance

This information is important to provide evidence that your safety assessment process appropriately considers publicly available toxicological data and the properties of any substances you have evaluated in context.

Wildtype Response: As described in CCC 000005, Wildtype undertook a component-by-component analysis of each substance used in the production of its cultivated salmon. Substances that were either permitted by federal regulation to be used in food without limitation or permitted to be directly added to food in a manner consistent with Wildtype's use were not subjected to further safety assessments.

The remaining 27 inputs were subjected to a safety assessment via mass balance calculation, and, in some cases, testing of harvested cells for the presence of the inputs demonstrated that the substances were not present in the finished product at or at levels greater than the limit of detection. Please see our answer to question 24 for two representative inputs that were tested and not detected in the finished product.

In general, all of the inputs described in Figure 14 in CCC 000005 were calculated to be trivially small, below the range of parts per trillion, and therefore practically not detectable in the finished product. The absence of meaningful exposure to these substances in the finished food product provides assurance that their use is safe. For completeness, however, the table below summarizes available scientific literature, when available, substantiating our general claim that the presence of cell culture medium constituents in the finished product are many orders of magnitude below established safety thresholds

found in the scientific literature (e.g., for all practical purposes, not present). Wildtype follows accepted convention in assuming that a margin of exposure of 100-fold or greater between the NOAEL and estimated dietary intake from food exposure is adequate to establish the safety of the substances below that are not commonly used in food production. Additionally, since submitting CCC 000005, we have refined our process, removing a number of inputs that are no longer necessary, including animal serum. These are listed separately in Figure 2a below. Inputs that are only used prior to the biomass production / cell proliferation stages (e.g. cell line development or the cryopreservation of cell banks) are listed separately in Figure 2b because they are not part of food production and have exposure levels orders of magnitude below those of all other inputs.

Figure 2 – Safety assessment for cell culture media constituents

Figure 2a – No longer used in Wildtype's process

Inputs no longer used				
Coenzyme	DNA component	Sugar	RNA component	Animal serum
DNA component	DNA component	Cofactor	Antioxidant	

Figure 2b – Inputs only used prior to cell proliferation / not used in food production

Input	Estimated Daily Intake (mg / day)	Estimated Daily Intake (mg / kg bw / day)	Example NOAEL[8], ADI[9], UL[10] Limit	Estimate of Margin of Exposure
pH indicator (phenol red)	2.29E-31	3.2E-33	30 µg / kg bw / day[11,12]	9.38E+31
Calcium chelator (EDTA)	6.2E-30	8.8E-32	2.5 mg / kg bw / day[13,14]	2.84E+31
Cryopreservation agent (DMSO)[15]	5.45E-1020	7.7E-1022	1000 mg / kg bw / day[16]	1.3E+1024

[8] No observed adverse effect level
[9] Acceptable Daily Intake
[10] Tolerable Upper Intake Level
[11] *In silico* approaches were used to estimate the potential toxicity and determine the appropriate toxicological threshold of concern (TTC), which is an approach considered applicable to low levels of impurities found in food and flavoring agents per EFSA, 2016 and Kroes et al., 2004: 2016. Review of the Threshold of Toxicological Concern (TTC) approach and development of a new TTC decision tree. EFSA Published March 10, 2016. EN-1006. 50 pp and Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J.G., Würtzen, G. 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Fd Chem. Toxicol. 42: 65-83.
[12] Low potential toxicity was identified using the Cramer classification paradigm (Cramer class I) (Toxtree Ver 3.1.0 software). This conclusion was also supported by the lack of positive genotoxicity prediction (Derek Nexus ver 6.2.0) therefore Cramer class I of 1800 µg/day (30 µg/kg bw/day) is considered protective of any potential health effects.
[13] EDTA calcium disodium salt (CAS 62-33-9), disodium EDTA (CAS 6381-92-6), and edetic acid (CAS 60-00-4) are used as a food additive at up to 800 ppm according to the condition of use described in 21 CFR 172.120 and 172.135, respectively. An acceptable daily intake (ADI) of 2.5 mg/kg bw/day was determined to be safe for use as calcium disodium EDTA (JECFA, 1986). a 90-day rat inhalation toxicity study (ECHA Edetic Acid 60-00-4; Decision # TPE-D-0000002405-80-05/F – 13 November, 2012)
ECHA. 2022. Edetic acid CAS 60-00-4. Dossier last modified October 25, 2022 and date access December 13, 2022.
[14] Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1974. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additives Series No. 5.
[15] Direct quantification is reported in the response to question 24.
[16] DMSO is listed in 21 CFR 172.859, 177.1655 and 177.244 as a flavoring agent or adjuvant and is also used as an excipient in a number of pharmaceutical preparations, including injectable. A NOAEL= 1000 mg/kg bw/day, the highest dose administered in a 28 days oral (gavage) rat study was reported in the literature (ECHA, 2022, SIDS, 2008). The NOAEL is based on the lack of reproduction and developmental toxicity screening test (OECD TG 421) and was used as a point of departure for the derivatization of a no effect level (DNEL) of 1.67 mg/kg bw/day for the general population long term exposure using an uncertainty factor of 600 was applied (ECHA, 2022). ECHA (2022) Dimethyl sulfoxide CAS 67-68-5. Dossier last modified October 13, 2022 and date access December 13, 2022.

Figure 2c – Non-protein inputs

Input	Estimated Daily Intake (mg / day)	Estimated Daily Intake (mg / kg bw / day)	Example NOAEL, ADI, UL Limit	Estimate of Margin of Exposure
Inorganic salt (Calcium Nitrate $4H_2O$)	3.27E-12	4.62E-14	3.7 mg / kg bw / day[17,18,19]	8.01E+13
Selenium	7.3E-16	1.03E-17	400 µg / day[20]	5.48E+14
Polysorbate (Tween 80)	7.1E-13	1E-14	25 mg / kg bw / day[21]	2.5E+15
Nicotinamide adenine dinucleotide	3.92E-13	5.54E-15	7.9mg/kg bw/day[22]	1.4E+15
Sugar (Glucuronate•Na)	1.02E-13	1.45E-15	GRAS[23]	N/A
Glucose metabolite (D-Glucuronolactone)	1.02E-13	1.45E-15	1000 mg / kg bw / day[24]	6.9E+17
Vitamin (Thiamine HCL)	3.38E-14	4.77E-16	22 µg / kg / day[25,26]	4.6E+13

[17] European Chemicals Agency EC number: 233-332-1 | CAS number: 10124-37-5 https://echa.europa.eu/registration-dossier/-/registered-dossier/15487/7/3/1 (accessed 15 Dec 2022) Acute oral toxicity in the rat oral LD_{50} was determined to be >300 mg/kg bw and >2000 mg/kg bw. Acute dermal and non-acute data uses read across Nitcal-K (potassium-pentacalcium-nitrate decahydrate CAS 905593-70-6). There is a lack of direct data or NOAEL established with the compound itself. An OECD 407 study with the read-across to substance Nitcal-K (potassium pentacalcium nitrate decahydrate) and an OECD 422 study with potassium nitrate did not show any adverse effects up to the highest dose level tested (1000 and 1500 mg/kg bw/day, respectively). JECFA established an ADI of 3.7 mg/kg bw/day for nitrate, the culprit of calcium nitrate toxicity.

[18] JECFA. 1995. Evaluation of certain food additives and contaminants. WHO Technical Report Series 859. 64 pp.

[19] https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/709 (data updated 2004) (accessed Dec 16 2022)

[20] Selenium functions through selenoproteins, several of which are oxidant defense enzymes. The Recommended Dietary Allowance (RDA) for selenium is based on the amount needed to maximize synthesis of the selenoprotein glutathione peroxidase, as assessed by the plateau in the activity of the plasma isoform of this enzyme. The RDA for both men and women is 55 µg (0.7 µmol)/day. The Tolerable Upper Intake Level (UL) for adults is set at 400 µg (5.1 µmol)/day based on selenosis as the adverse effect (IOM, 2000) . EPA IRIS summary identifies the Critical effect of Clinical selenosis and uses the "Conversion Factors: NOAEL (0.853 mg/day) and LOAEL (1.261 mg/day) calculated from regression analysis (log Y = 0.767log X - 2.248, where Y = blood selenium and X = selenium intake) as detailed in Yang et al. (1989a) based upon the correlation (r = 0.962) between dietary selenium intake and blood selenium level for data showing incidence of clinical selenosis in adults based on an average adult body weight of 55 kg (Yang et al., 1989b)" to calculate the Oral RfD of 0.005 mg/kg bw/day. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US); 2000. PMID: 25077263. DOI: 10.17226/9810.

[21] Tween 80 (AKA polyethylene glycol sorbitan monooleate CAS 9005-65-6) is used in a number of food applications including emulsifier and thickening agent (food additive) (21 CFR 73.1, 73.1001, 172.515, 172.836, 172.838, 172.840, 172.842, 173.340, 175.105, 176.180 and 178.3400). JECFA determined an ADI of 25 mg/kg bw (JECFA, 1973) Joint FAO/WHO Expert Committee on Food Additives. 1973. Toxicological evaluation of certain food additives with a review of general principles and of specifications. Technical report series No. 539.

[22] See references in CCC 000005

[23] Sodium glucuronate or glucuronic acid (CAS 576-37-4) is a sugar acid derived from glucose and is a precursor of vitamin C synthesis. It is naturally occurring in the human body and is involved primarily in the detoxification of xenobiotic in the process known as glucuronidation. The latter uses UDP-glucuronic acid (glucuronic acid linked via a glycosidic bond to uridine diphosphate) as an intermediate. UDP-glucuronic acid is formed in the liver of all animals and excreted in the urine where it was first identified. Sodium glucuronate or glucuronic acid is found naturally in food and used in a variety of non-food applications e.g., cosmetic ingredient (skin conditioning agent). Glucuronic acid is structurally similar to glucose (CAS 50-99-7) (PubChem) which is considered GRAS (21 CFR 1841.1857) and is listed in the Food Chemicals Codex as used in food.

[24] D-Glucuronolactone is a naturally occurring substance that is an important structural component of nearly all connective tissues. It is sometimes used in energy drinks. EFSA has concluded that it is unlikely that glucurono-γ-lactone would have any interaction with caffeine, taurine, alcohol or the effects of exercise. In a scientific opinion paper on the safety of the use of glucurono-γ-lactone in energy drinks in 2009. The NOAEL cited was from a 13-week oral gavage toxicity study of D-glucurono-γ-lactone in rats, with specific focus on the kidneys. This study used the same rat strain as the previous study reported in the SCF Opinion of 2003. Extensive urinalysis and histopathological examinations demonstrated no treatment-related effects. Based on the results of this study, the NOAEL for daily oral administration of D glucurono-γ-lactone in rats was 1000 mg/kg bw/day, the highest dose tested. EFSA. 2009. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on a request from the Commission on the use of taurine and D-glucurono-γ-lactone as constituents of the so called "energy" drinks. EFSA J. 935: 1-31. https://doi.org/10.2903/j.efsa.2009.935

[25] Thiamine hydrochloride is considered an essential nutrient in the human diet and is considered GRAS when used as flavoring agent, adjuvant or food supplement by the FDA (21 CFR 184.1875). The estimated dietary intake of thiamine hydrochloride (No 1030; JECFA 2002) (FEMA No 3322) from its use as flavoring agent in Europe and in the US is very low with values of 48 and 22 µg/ kg bw/day, respectively, which are more than 500 and 1000 times, respectively lower compared to the NOEL of 36 mg/kg bw/day established in a 90-day dietary rat study. Therefore, JECFA (2002) did not establish an ADI due to the lack of safety concerns when used as a food additive. However, the reference for this study was not found in the JECFA review. ECHA. 2022. Thiamine hydrochloride CAS 67-03-8. Dossier last modified March 16, 2020. Date access December 14, 2022.

[26] Joint FAO/WHO Expert Committee on Food Additives. 2002. Evaluation of certain food additives. WHO Technical Report Series 913.

Input	Estimated Daily Intake (mg / day)	Estimated Daily Intake (mg / kg bw / day)	Example NOAEL, ADI, UL Limit	Estimate of Margin of Exposure
Vitamin (Thiamine monophosphate)	1.85E-14	2.62E-16	4.89 mg / day[27] / 100 mg/day[28]	2.64E+14
Vitamin (Thiamine diphosphate cocarboxylase)	5.67E-14	8.01E-16	4.89 mg / day[29] / 100 mg/day[30]	8.62E+13
Taurine	2.4E-13	3.39E-15	0.45 mg/kg bw/day[31]	1.3E+12

Figure 2d – Proteins and DNA bases

Input	Estimated Daily Intake (mg / day)	Estimated Daily Intake (mg / kg bw / day)	Safety assessment summary
DNA component (2'-Deoxycytidine•HCl)	5.67E-13	8.01E-15	DNA bases are digested and naturally anabolized into cellular DNA or catabolized according to described physiological pathways.[32, 33] All proteins listed here (insulin, transferrin, and fibroblast growth factor) have an estimated daily intake orders of magnitude below that of physiological activity; the ingestion of growth factors is addressed in the response to question 8 below, using FGF2 as an example.
DNA component (5'-Methylcytosine • HCl)	5.45E-15	7.7E-17	
Functional protein (insulin)	1.09E-12	1.54E-14	
Functional protein (transferrin)	6E-13	8.47E-15	
Functional protein (fibroblast growth factor)[34]	1.09E-16	1.54E-18	

No human oral intake information or history of safe use in the literature is available for the compounds listed above. Additionally, no information on the toxicity of these compounds is available in the public literature. However, protein and nucleic acids are ubiquitous in the human diet and are components of the human body, essential to sustain life. Data from proteins in general are available and indicate complete breakdown in the gastrointestinal tract by proteolytic enzymes to smaller oligopeptides, peptides, and amino acids with little to no potential for systemic bioavailability of protein (< 1% oral absorption).[35] The combined action of the proteolytic enzymes of pancreatic secretions and intestinal mucosa results in rapid and further digestion. As such, proteins possess no potential for systemic

[27] National Institutes of Health (NIH) Office of Dietary Supplements (ODS). 2021. Factsheet on Thiamine. Last updated March 2021. Accessed 19 Dec 2022.
[28] Thiamine monophosphate is naturally present in the human diet and is an important source of vitamin B_1. Estimated food intake of vitamin B_1 (97.5th percentile) in some European countries varied from 1.90 mg/day to 6.35 mg/day. In US adults aged 20 and older, the average daily thiamin intake from foods and supplements is 4.89 mg in men and 4.90 mg in women (NIHODS 2021). EFSA (2008) evaluated the use of thiamine monophosphate as a food supplement and determined that there is no safety concern when use at up to 100 mg/ day. EFSA. 2008. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS). Benfotiamine, thiamine monophosphate chloride and thiamine pyrophosphate chloride, as sources of vitamin B_1 added for nutritional purposes to food supplements. EFSA J 864: 1-31.
[29] Thiamine diphosphate also known as cocarboxylase is the active form of vitamin B_1 and an important dietary supplement. Estimated food intake of vitamin B_1 (97.5th percentile) in some European countries varied from 1.90 mg/day to 6.35 mg/day. In US adults aged 20 and older, the average daily thiamine intake from foods and supplements is 4.89 mg in men and 4.90 mg in women (NIHODS 2021). EFSA (2008) evaluated the use of thiamine diphosphate as a food supplement and determined that there is no safety concern when use at up to 100 mg/day corresponding to 1.7 mg/kg bw/day. National Institutes of Health (NIH) Office of Dietary Supplements (ODS). 2021. Factsheet on Thiamine. Last updated March 2021. Accessed 19 Dec 2022.
[30] EFSA. 2008. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS). Benfotiamine, thiamine monophosphate chloride and thiamine pyrophosphate chloride, as sources of vitamin B_1 added for nutritional purposes to food supplements. EFSA J 864: 1-31.
[31] See references in CCC 000005
[32] Liu Y, Zhang Y, Dong P, An R, Xue C, Ge Y, Wei L, Liang X. Digestion of Nucleic Acids Starts in the Stomach. *Sci Rep.* 2015 Jul 14;5:11936. doi: 10.1038/srep11936. PMID: 26168909
[33] Hill JM, Morse PA Jr, Gentry GA. Metabolism of deoxycytidine, thymine, and deoxythymidine in the hamster. *Cancer Res.* 1975 May;35(5):1314-9. PMID: 1120315.
[34] Direct quantification is reported in Figure 3, in response to question 8 of this document.
[35] Renukuntla, J., Vadlapudi, A.D., Patel, A., Boddu, S.H.S., Mitra, A.K. 2013. Approaches for enhancing oral bioavailability of peptides and proteins. Int. J. Pharm. 2013 447(0): 75–93.

toxicity and are not normally associated with adverse effects.[36,37] Similarly, DNA and RNA consumed from food are metabolized in the digestive tract by endonucleases, phosphodiesterases and nucleoside phosphorylases into oligonucleotides, nucleotides and free bases.[38] Some of these metabolites can be absorbed from the gastrointestinal tract and utilized for the salvage synthesis of nucleic acids by the human body.

Three sample calculations for these values are provided in the response to question 24. Margin of exposure is calculated by dividing NOAEL, ADI, UL by the estimated daily intake.

6: Safety Assessment - Information Requested

On page 31 of the disclosable safety narrative, you state that "The conclusion of this analysis was that all 27 of these inputs either are not present in the final product at meaningful levels, or are present only inadvertently and at very low concentrations". On page 38 you state that "… the 27 inputs in question ranged from 3.27 x 10-9 µg per day to 5.45 x 10-7154 µg per day in the finished product and therefore not present in the final product at meaningful levels". Please provide, for addition to the disclosable safety narrative, some additional discussion to clarify your perspective on the significance (if any) of the distinction between "very low concentrations" and "not present at meaningful levels" as it pertains to safety. You may find it helpful to incorporate some discussion of reference levels and margins of exposure noted in the prior question.

Significance
This information is important to demonstrate that you are appropriately considering whether your safety assessment fully addresses the full range of possible exposure for individual substances used in the cell culture process given the variety of substances and uses involved.

Wildtype Response: As Figure 1 above shows, the 27 inputs are present at levels below levels of detection of any available analytical methods, and in addition are orders of magnitude smaller than NOAEL levels documented in the literature. For practical purposes, these substances are not present in the final product. Therefore, there is no distinction between our use of the terms "very low concentrations" and "not present at meaningful levels" as it pertains to safety. Both terms employed in CCC 000005 are interchangeable and can be understood to mean that the substances are not present in practical terms in Wildtype salmon.

7: Safety Assessment - Information Requested

On page 45 of the disclosable safety narrative, you discuss the stability of fibroblast growth factor 2 (FGF2). You indicate that similar considerations and a similar stability profile apply to other

[36] Delaney, B., Astwood, J.D., Cunny, H., Eichen Conn, R., Herouet-Guicheney, C., MacIntosh, S., Meyer, L.S., Privalle, L., Gao, Y., Mattsson, J., Levine, M. 2008. Evaluation of protein safety in the context of agricultural biotechnology. Food Chem. Toxicol. 46 (Suppl. 2): S71-S97.
[37] Sjoblad, R. D. McClintock, J.T., Engler, R. 1992. Toxicological considerations for protein components of biological pesticide products. Reg. Tox. Pharmacol. 15 (1): 3-9.
[38] Liu, Y., Zhang, Y., Dong, P., An, R., Xue, C., Ge, Y., Wei, J., Liang, X. 2015. Digestion of nucleic acid starts in the stomach. Nature 5: 11936.

protein-based growth factors. Please provide, for addition to the disclosable safety narrative, your basis for drawing this conclusion, including relevant citations to the scientific literature, if applicable.

Significance
This information is useful to clarify the extent to which your safety assessments for these substances are able to draw on relevant information about stability, activity, and prior exposure for endogenous forms of these proteins. For example, in the scientific literature, growth factors with varying stability in aqueous solutions utilize specific receptor/signaling pathways whose magnitude, duration, and mode of action may differ from each other. Thus, an overly generalized safety narrative based on one growth factor may not be sufficient without context.

Wildtype Response: Although different protein growth factors initiate widely varied physiological effects, they share structural similarities with all other proteins that determine their stability in cell culture and their ability to function as signaling molecules. The relevant fundamental characteristics that determine stability and activity over time are: conformational stability in aqueous solution, predisposition to hydrolysis (or other mechanism of protein digestion), pH sensitivity, and thermal sensitivity.[39]

Similarities between the proteins with respect to these characteristics have been extensively detailed in the literature for fibroblast growth factor 1 (FGF1), FGF2, transforming growth factor-beta1 (TGFβ1), and interferon beta (IFN-β), among others.[40] In cases where primary literature was not available to directly confirm the reactivity and degradation mechanisms of protein growth factors, amino acid sequences and protein structures were used to predict these characteristics in the same reference for epidermal growth factor (EGF), glucagon, growth hormone, insulin, insulin-like growth factor 1 (IGF1), vascular endothelial growth factor (VEGF), and numerous others.[41]

Collectively, these studies demonstrate that, while the mechanism of activity, dose-dependence, and kinetics of activity all vary across protein growth factors, these proteins have one important unifying characteristic: enhanced degradation with increased temperature. From a safety perspective, this is the salient feature of protein growth factor stability because Wildtype's production process includes a thermal inactivation step. Thus, even if growth factors were to be present, they would be inactivated prior to consumption.

One of many relevant examples supporting this conclusion is insulin, the degradation pathways of which have been extensively elucidated.[42] The left panel below (from the prior cited reference) demonstrates the degradation kinetics as a function of temperature; the y-axis shows the accumulation of degradation products over time (x-axis):

[39] Pearlman, R. and Wang, YJ (2002). Formulation, Characterization, and Stability of Protein Drugs: Case Histories. *Pharmaceutical Biotechnology*. doi: 10.1007/b112935
[40] Ibid.
[41] Ibid.
[42] Brange J, Langkjaer L, Havelund S, Vølund A. Chemical stability of insulin. 1. Hydrolytic degradation during storage of pharmaceutical preparations. *Pharm Res.* 1992 Jun;9(6):715-26. doi: 10.1023/a:1015835017916. PMID: 1409351.

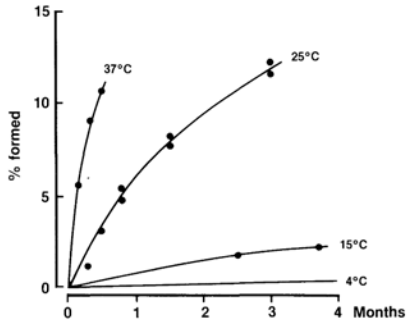


Fig. 7. Time courses of formation of the split product (A8–A9) during storage of bovine insulin zinc suspension, crystalline at different temperatures. The course at 4°C, based on results (n = 6) from 2 to 10 years of storage, corresponds to the formation of approx. 0.05% per month.

Table III. Shelf-Life Estimates (T90; 0.105/k_{obs}) for hEGF 1-48 at pH 6.0 (0.02 M Sodium Phosphate/0.01% Polysorbate 80)

Temperature (°C)	Observed[a] shelf-life (days)	Predicted[b] shelf-life (days)
45	1.0	1.0
30	5.9	6.1
10	91.2	90.4
8	****	120.9
2	****	296.7

[a] Shelf-life estimated directly from the apparent first-order rate constant determined from the log-linear least squares fits for loss of hEGF 1-48 vs. time.
[b] Shelf-life estimated from the apparent first-order rate constant predicted from the Arrhenius relationship (ln k_{obs} vs. 1/T; E_a = 22.99 kcal/mol, A = 34.10).

As shown in the left panel, insulin degradation kinetics are profoundly accelerated with increasing temperature, and the highest temperature measured (37˚C) still does not approach that of Wildtype's thermal inactivation step. The right panel, from Senderoff et al.'s characterization of epidermal growth factor (EGF) stability,[43] shows a similar increase in degradation rate with a completely different growth factor. Finally, Wildtype has demonstrated undetectable levels of thermostable FGF2 in the final product (Figure 3, in response to question 8 below), confirming that the low initial concentration of protein growth factor, subsequent dilutive steps, and thermal inactivation, result in undetectable levels of active protein growth factor in the final product.

8: Safety Assessment - Information Requested

On page 45 of the disclosable safety narrative, you discuss the stability of FGF2. Stability and persistence of growth factors in cell culture is a long-standing technical challenge. This issue and some potential strategies to address it are discussed in the scientific literature.[44] Please provide confirmation that your use of FGF2 and other similar growth factors does not involve methods that enhance the stability or activity of these proteins, and that the protein sequences have not been modified for either purpose compared to endogenous forms present in agriculturally relevant species. For any instance where this does not apply, please discuss whether these modifications would have any significance from a food safety perspective.

Significance
This information is useful to clarify the extent to which your safety assessments for these substances are able to draw on relevant information about stability, activity, and prior exposure for endogenous forms of these proteins.

[43] Senderoff RI, Wootton SC, Boctor AM, Chen TM, Giordani AB, Julian TN, Radebaugh GW. Aqueous stability of human epidermal growth factor 1-48. *Pharm Res*. 1994 Dec;11(12):1712-20. doi: 10.1023/a:1018903014204. PMID: 7899233.
[44] For example, Dvorak, P. et al. (2018). Computer-assisted engineering of hyperstable fibroblast growth factor 2. *Biotechnology and Bioengineering*, 115(4), p. 850-862. doi: 10.1002/bit.26531

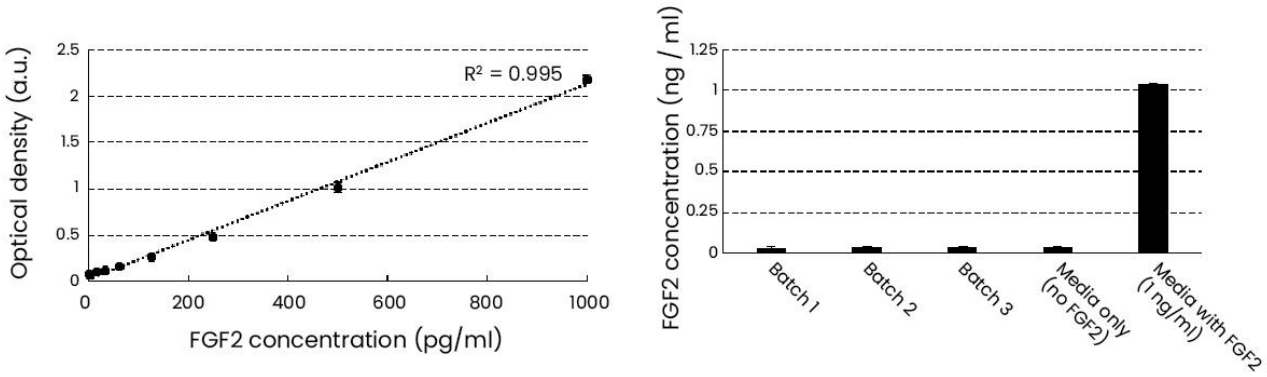
Wildtype Response: As correctly noted here, the stability of protein growth factors such as FGF2 is a major determinant of the efficiency and reproducibility of cell culture.[45] In order to maintain culture consistency and reduce the number of growth factor additions during cell culture, Wildtype has adopted the use of FGF2 with enhanced thermal stability (i.e. decreased predisposition toward spontaneous denaturation in the temperature range of normal cell culture). The following discussion summarizes the significance of this in the context of food safety.

As noted in CCC 000005, successive wash steps bring the final estimated concentration of growth factors such as FGF2 down to the range of 1E-15 mg/L. Exposure to common proteases typically found in cell culture[46, 47, 48] would also naturally degrade remaining intact FGF2. As shown in figure 4a of the cited paper,[49] the thermal transition midpoint (T_m , or the equilibrium point between active and inactivated thermostable FGF2) is in the range of Wildtype's thermal inactivation step (65-70˚C), indicating that at least part of any remaining thermostable FGF2 would be even further neutralized during this stage. It is also worth noting that the safety discussion on page 47 of CCC 000005 applies in this context, as it demonstrates the absence of side effects or pathologic sequelae when patients ingested modified stable FGF2 at doses trillions of times higher than that predicted to exist in the finished product.[50]

Despite this, the most compelling evidence surrounding the safety of thermostable FGF2 is the direct measurement of its concentration in the finished product by enzyme-linked immunosorbent assay (ELISA) that has been validated for its intended use. As shown in the left panel of Figure 3, we first generated a standard curve to demonstrate the range of sensitivity for this method; the lower limit of detection was determined to be 16 pg/ml (R^2 = coefficient of determination; a.u. = absorbance units). In the right panel, thermostable FGF2 was measured in the final product of three non-consecutive batches (the same batches used for adventitious agent testing in Figure 6). Cell culture media with freshly added thermostable FGF2 (1 ng/ml) was used as a positive control (rightmost bar), and cell culture media with no FGF2 added was used as a negative control (second bar from the right). As shown in the right panel, all three final product batches and the negative control demonstrated undetectable levels of thermostable FGF2. Error bars represent the standard deviation for all technical replicates in each condition.

[45] 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. doi: 10.1002/stem.1021.
[46] Elliott, P., Hohmann, A. & Spanos, J. Protease expression in the supernatant of Chinese Hamster Ovary cells grown in serum-free culture. *Biotechnology Letters* 25, 1949–1952 (2003). https://doi.org/10.1023/B:BILE.0000003992.09492.4b
[47] Busby, W. H., Nam, T. J., Moralez, A., Smith, C., Jennings, M., & Clemmons, D. R. (2000). The complement component C1s is the protease that accounts for cleavage of insulin-like growth factor-binding protein-5 in fibroblast medium. *Journal of Biological Chemistry*, *275*(48), 37638-37644.
[48] Ikonomou, L., Peeters-Joris, C., Schneider, Y. J., & Agathos, S. N. (2002). Supernatant proteolytic activities of High-Five insect cells grown in serum-free culture. *Biotechnology letters*, *24*(12), 965-969.
[49] Dvorak, P. et al. (2018). Computer-assisted engineering of hyperstable fibroblast growth factor 2. *Biotechnology and Bioengineering*, 115(4), p. 850-862. doi: 10.1002/bit.26531
[50] Hull MA, Knifton A, Filipowicz B, Brough JL, Vautier G, Hawkey CJ. Healing with basic fibroblast growth factor is associated with reduced indomethacin induced relapse in a human model of gastric ulceration. Gut. 1997 Feb;40(2):204-10. doi: 10.1136/gut.40.2.204. PMID: 9071932

Figure 3 – Quantification of thermostable FGF2



9: Microbial and Viral Testing - Information Requested

On page 12 of the disclosable safety narrative, you state "Upon receipt of inputs needed for the downstream production process, such as powdered cell culture media and scaffold inputs, Wildtype's production staff validates contents and quality attributes, including aerobic plate counts". Please provide, for addition to the disclosable safety narrative, the method used, and the specification for the aerobic plate count analysis that occurs at this step.

Significance
Specifications are an important element of identity and provide assurance of a performance standard for the control of certain contaminant risks.

Wildtype Response: Microbial testing is not a requirement for all inputs; we adopt a risk-based approach to testing and validating inputs. Standard aerobic plate counting carried out by an accredited laboratory represents one such test, using a validated and proprietary method.[51] The specification for aerobic plate counts are <1,000 colony forming units (cfu) per gram (see additional detail in Figure 5).

Wildtype's quality and food safety program assigns required testing in raw material specifications (RMS) for each input or processing aid in our production process. Example tests and criteria include appearance (via visual inspection), certificate of inspection examination, size confirmation, and label verification. In all cases, testing used is validated for its intended purpose, and carried out by accredited laboratories.

10: Scaffold Production - Information Requested

On page 14 of the disclosable safety narrative, you state "Inputs for Wildtype's scaffolds are gathered by operators, quality-checked, mixed, and assembled. Inputs are then sterilized using techniques such as heating or ethanol treatment, and are seeded with cells under aseptic conditions." For addition to the

[51] Exact Scientific Services method ESS_2.3.5.b* Exact Scientific Services is an ISO 17025 ANAB Accredited Laboratory: Cert. #: AT-1754

disclosable safety narrative, please specify whether the sterilization procedures are validated for their intended purpose.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including characterization of the final product.

Wildtype Response: Two criteria are used to validate the intended use of sterilization methodologies as they apply to scaffolds: sterilization efficacy and the preservation of scaffold integrity. In the case of heating, protocols are established to preserve scaffold integrity (verified by post-sterilization scaffold textural analysis and cell seeding / integration studies) and achieve microbial sterilization (verified by visual inspection after 72h in cell culture media, per standard protocol[52] validated for the purpose of assessing scaffold sterility). Wildtype's sterilization methodologies do not deviate from standardized protocols, which have been extensively validated for scaffolds (reviewed by Dai et al.)[53]

11: Scaffold Production Information Requested

On page 18 of the confidential supplementary material, you note that eight substances are used in the production of the scaffold. We recognize that such formulations may continue to evolve over time. However, for addition to the disclosable safety narrative, please provide some additional information about the eight substances currently anticipated to be used in the production of the scaffold, as well as an estimate of the amount of each substance that is expected to be present in the harvested cellular material.

Significance
This information will help FDA more clearly convey the identity of the product described in CCC 000005 at harvest when documenting our evaluation of your submission.

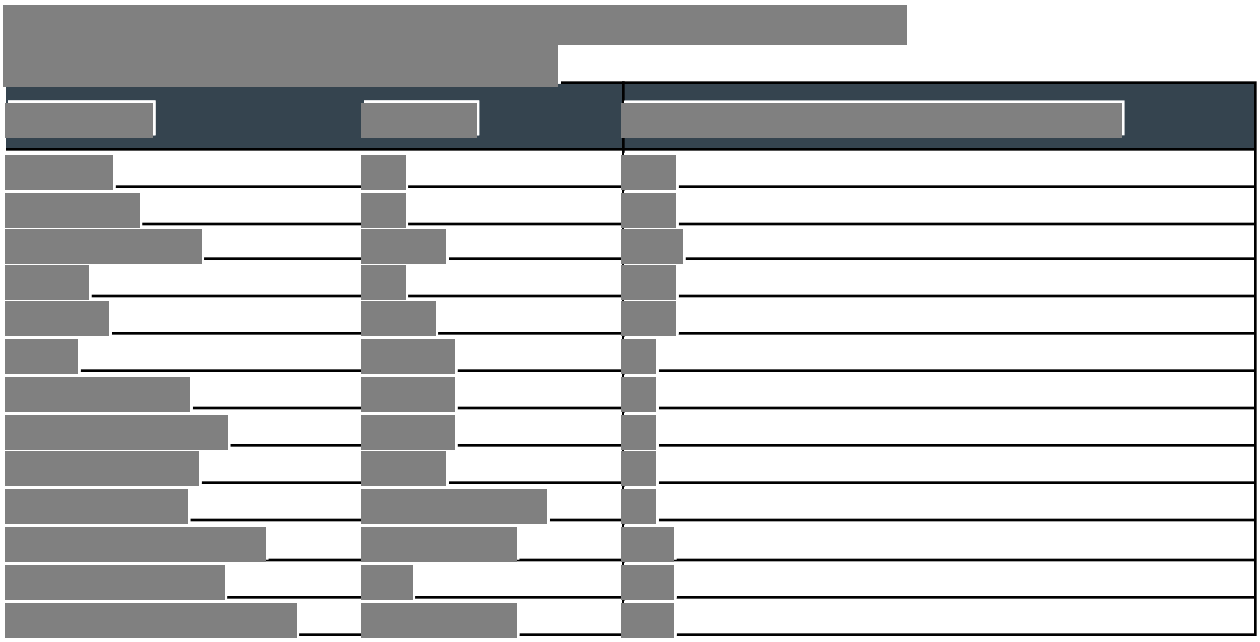
Wildtype Response: Wildtype's scaffold formulation has been updated since CCC 000005 was submitted in June 2022. Figure 4 below summarizes the eight classes of substances (excluding water) used in production of Wildtype's scaffolds as well as ranges for inclusion in the finished product.

[52] Łopianiak I, Butruk-Raszeja BA. Evaluation of Sterilization/Disinfection Methods of Fibrous Polyurethane Scaffolds Designed for Tissue Engineering Applications. Int J Mol Sci. 2020 Oct 30;21(21):8092. doi: 10.3390/ijms21218092. PMID: 33142959 [Method described in Section 4.3.1]
[53] Dai Z, Ronholm J, Tian Y, Sethi B, Cao X. Sterilization techniques for biodegradable scaffolds in tissue engineering applications. J Tissue Eng. 2016 May 17;7:2041731416648810. doi: 10.1177/2041731416648810. PMID: 27247758; PMCID: PMC4874054.

Figure 4a – Scaffold inputs

Scaffold Input Class	Examples	Estimate of amount in finished product as % of total weight
1. Fats	Polyunsaturated, monounsaturated	0-15%
2. Proteins	Enzymes, lentil, pea, soy, wheat, yeast extract	0-10%
3. Sugars	Corms, glucose, galactose, maltose	0-5%
4. Starches	Arrowroot, corn, kuzu, tapioca	<1%
5. Gums	Carrageenan, cellulose, lecithin, guar, gellum	<1%
6. Salts	Sodium chloride, potassium chloride, monopotassium glutamate, monosodium glutamate, sodium bicarbonate, calcium chloride	<1%
7. Natural flavors	-	<1%
8. Natural pigments	-	<1%

START OF CONFIDENTIAL DISCLOSURE[54]



END OF CONFIDENTIAL DISCLOSURE

Cell Culture Process

12: Cell Growth in Bioreactor - Information Requested

On page 13 of the disclosable safety narrative, you state "After one to four weeks of growth in a bioreactor, cells are harvested. Agitation in the bioreactor ceases and cells are allowed to settle. Supernatant is removed from the tank using peristaltic pumps. Cells are then centrifuged, collected, and quality tested. At the completion of a batch, the bioreactor is taken apart, sterilized using a combination of clean-in-place, steam-in-place, and clean-out-of-place techniques, reassembled,

[54] Trade secret and confidential commercial information exempt from disclosure under exemption 4 of the Freedom of Information Act

tested, and prepared for the next run". Please describe, for addition to the disclosable safety narrative, what type of testing is performed at this stage. Please describe whether there are specification parameters, including specifications for adventitious agent testing, for the harvested cellular material at this stage.

Significance
Specifications are an important element of identity and provide assurance of a performance standard for the control of certain contaminant risks. Additionally, this information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture.

Wildtype Response: Prior to harvesting a bioreactor, a sample is taken and both the viable cell density and viability percentage are recorded. The specification for accepting the product is cell viability ≥80%. These characteristics do not represent critical controls with respect to food safety; rather, aberrations in either value signify the potential presence of adventitious agents that affect cell growth. For this reason, testing for adventitious agents (aerobic bacteria, molds, yeast, *E. coli*, coliforms, and *S. aureus*) is initiated if cell growth does not correspond to historical patterns established at both the benchtop and pilot-scale bioreactors, or if viable cell density is <80%. Specifications for testing are the same as those enumerated in Figure 5.

13: Cell Growth in Bioreactor - Information Requested

For addition to the disclosable safety narrative, please describe the process controls and management systems employed when cells do not display an expected growth profile and unintended effects of immortalization.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture
Hazard Analysis and Preventive Controls

Wildtype Response: Wildtype monitors several phenotypic stability parameters during production to assess for deviations in cell growth and behavior. The first is the rate of cellular growth, which can be expressed as the change in viable cell density over time or as the doubling time. In the cell proliferation stages (prior to addition to scaffolds), cells are seeded in bioreactors at defined starting densities (expressed as the number of cells per mL), providing a baseline for each run. Viable cell density is also measured directly at the conclusion of each run, and at predefined intervals throughout. These data provide a highly accurate and reproducible estimate of cellular growth over time in each batch. A deviation of ±35% or more from the average expected growth rate (characterized during historic runs at various scales) or a cell viability <80% represents a significant aberration; testing for adventitious agents is initiated in either case, and lots found to be positive for tested contaminants are rejected for

further processing, according to the plan described in the response to question 12. Of note, a spontaneous increase in growth rate by ≥35% is considered highly unlikely, given both historical data from Wildtype's cell lines and the tightly controlled physiology underlying fish cell division (best characterized in zebrafish[55, 56]).

In both the cell proliferation and maturation stages of production, phenotypic stability is assessed by a second parameter: cellular metabolism. Because shifts in cell nutrient consumption patterns often reflect changes in underlying cellular physiology, metabolic profiling is used to ensure ongoing cell culture reliability and safety. In its simplest form, metabolic profiling encompasses the measurement of key nutrients and waste products over time to assess the physiological state of cells. For example, the rate of oxygen consumption, sugar metabolism, amino acid incorporation, and lactic acid production are predictable during the course of cell proliferation or maturation. For this reason, pH is continuously measured in Wildtype production cultures. Spontaneous pH changes >0.5 over the course of 24 hours represent aberrations that prompt culture sampling, the quantification of viable cell density, quantification of lactic acid, lactate dehydrogenase, glucose, microscopic observation to identify adventitious agent growth, and quantitative third-party testing for the adventitious agents described in the response to question 12. Although this approach serves to identify rapid deviations in culture conditions and mitigate immediate safety risks, longer-term changes to culture result from more insidious changes over time. For this reason, production run data (including viable cell density, cell viability, DO, and pH) are graphed over time and compared with historical data, including those used to validate cell banks. When statistically significant deviations are noted (one standard deviation for viable cell density, cell viability, and DO) at any point in the run, cultures are terminated and the seed train is initiated from a new working cell bank.

To date, Wildtype has been unable to identify a scientific report describing pathogenesis resulting from the immortalization of animal cells in culture. Nonetheless, the described approaches have been implemented to monitor production for theoretically deleterious consequences of this process.

14: Cell Growth in Bioreactor - Information Requested

On page 17 of the disclosable safety narrative, Figure 12 lists the potential hazards identified at the source cell procurement, cell line establishment, and cryobanking stages, including "Potential presence of microflora or bacterial pathogens, viruses, and parasites in donor animals during cell isolation", and provides justification as to why a preventive control is not needed, "Parasite introduction mitigated by isolating cells from eggs and via sterile filtration". On page 10 of the disclosable safety narrative, you mention that the musculature of embryonic or juvenile fish is used for cell isolation. Please provide, for addition to the disclosable safety narrative, the mitigation strategy employed for the cells isolated from other sources, including alevin and fry.

[55] Duffy KT, McAleer MF, Davidson WR, Kari L, Kari C, Liu CG, Farber SA, Cheng KC, Mest JR, Wickstrom E, Dicker AP, Rodeck U. Coordinate control of cell cycle regulatory genes in zebrafish development tested by cyclin D1 knockdown with morpholino phosphorodiamidates and hydroxyprolyl-phosphono peptide nucleic acids. *Nucleic Acids Res.* 2005 Sep 2;33(15):4914-21. doi: 10.1093/nar/gki799. PMID: 16284195

[56] Sugiyama M, Sakaue-Sawano A, Iimura T, Fukami K, Kitaguchi T, Kawakami K, Okamoto H, Higashijima S, Miyawaki A. Illuminating cell-cycle progression in the developing zebrafish embryo. Proc Natl Acad Sci USA. 2009 Dec 8;106(49):20812-7. doi: 10.1073/pnas.0906464106. Epub 2009 Nov 18. PMID: 19923430

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.
Further, this information strengthens the "Hazard Analysis and Preventive Controls" table presented in Figure 12.

Wildtype Response: Potential biological hazards for cells isolated from both eggs and juvenile fish originating from hatcheries include those transmitted by human handling, environmental hazards, and transmission from the donor fish into cell culture such as *Listeria monocytogenes, Salmonella spp., Staphylococcus aureus,* and *E. coli*.

Each of these potential pathogenic risks is mitigated by a set of controls employed by Wildtype personnel. These include aseptic and cGMP techniques such as the use of laminar flow hoods, gloves, and gowning techniques. Sterile rinsing of the donor fish tissue using hydrogen peroxide significantly reduces the potential for transmission of the aforementioned potential pathogens. As described in CCC 000005, antibiotics are used for the first several passages in order to further mitigate the growth of adventitious agents in culture. Additionally, in these early stages, cell cultures are monitored carefully via microscope and for pH changes that result from contamination. Cultures displaying the presence of adventitious agents or spontaneous pH changes >0.5 over the course of 24 hours are terminated. All stages of production from cell culture until final processing take place within a cGMP compliant facility to mitigate the risk of human pathogenic transmission. Finally, a terminal thermal step at the end of Wildtype's production process further mitigates the risk of any potential contamination, and routine lot testing for the presence of potential pathogens (see further discussion in response to question 15 below) helps to ensure that controls employed earlier in the production process, including at the cell line isolation step, are effective.

15: Cell Growth in Bioreactor - Information Requested

On page 21 of the disclosable safety narrative, Figure 12 lists the potential hazards identified at the cell maturation stage (also discussed on pages 28-30 of the disclosable safety narrative), including "Survival of potential pathogens", and identifies a process preventive control as "Product is heated to lethal temperature". Biogenic amines and toxins are not included in the list of potential hazards identified at this stage; for example, members of the Enterobacteriaceae family may produce biogenic amines (histamine is discussed below), while Staphylococcus aureus may produce heat resistant toxins and is identified as a potential pathogen of concern in your production process. Further, there are reports in the literature that growth of S. aureus and production of staphylococcal enterotoxins may be decoupled (i.e., active growth of S. aureus is may not be necessary for enterotoxin production), while foodborne illness attributed to S. aureus is often associated with growth in protein-rich food, including

fish and fish products.[57,58] Please describe whether the production of microbial toxins is a safety concern in your production process, particularly those that may survive the heat treatment step and persist during cell maturation.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants. Further, this information strengthens the "Hazard Analysis and Preventive Controls" table presented in Figure 12.

Wildtype Response: The potential generation of microbial toxins and biogenic amines is addressed by direct measurement of both classes of toxins. As described in Figure 5 below, Wildtype (via accredited third-party laboratories and using established methods that are validated for their intended purposes) has initiated a three part testing program. First, in response to question 23 below, and detailed in Figure 6, an extensive panel of pathogen testing on three non-consecutive batches of Wildtype salmon was conducted, encompassing biogenic amines and microbial toxins. Second, a more limited panel, specifically focused on common pathogens (including *E. coli*, *Enterobacteriaceae*, *Salmonella*, and *Listeria*), will be pursued biweekly over an initial 90-day period (baseline testing) to achieve a statistically significant assessment of pathogenic risk. Following this 90-day period, a more limited panel to test production lots will be pursued; this panel will exclude two less likely contaminants (based on previous Wildtype testing), *Bacillus cereus* and *Campylobacter spp.* if these tests are not positive during baseline testing, and will also exclude coliforms while continuing the more comprehensive test for *Enterobacteriaceae* (still encompassing coliform testing). These tests will be performed on an ongoing basis (at least twice per year) based upon the results of our risk-based hazard analysis and preventive control plan as described in CCC 000005.

Specifications for each potential hazard have been set according to a risk-based approach, set to below the limit of detection or to levels typically found in conventional salmon. Where available, relevant citations for ready-to-eat fish products are provided in the Specifications column of Figure 5. Specifications for tests with a high likelihood of acute pathogenesis (*Campylobacter*, *Salmonella*, *Listeria*, *Staphylococcus* enterotoxin, and *Clostridium perfringens* toxin) are set at the limit of detection for the referenced assays.

Additionally, Wildtype's practices of aseptic technique, documentation, and employing good manufacturing practice create an environment where the potential for introduction of pathogens is significantly minimized.

[57] Schelin, J. et al. (2011). The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. Virulence, 2(6), p. 580-592. doi: 10.4161/viru.2.6.18122
[58] Grispoldi, L. et al. (2021). Staphylococcus aureus enterotoxin in food of animal origin and staphylococcal food poisoning risk assessment from farm to table. Italian Journal of Animal Science, 20(1), p. 677-690. doi: 10.1080/1828051X.2020.1871428

Figure 5 – Finished product testing

Potential Hazard	One time	Baseline	Ongoing	Method	Specification
Aerobic plate count	x	x	x	Plate count[59]	<1,000 cfu/g[60]
Yeast/mold	x	x	x	Plate count[61]	<100 cfu/g
Enterobacteriaceae	x	x	x	Plate count[62]	<100 cfu/g[60]
Coliforms	x	x		Plate count[63]	<100 cfu/g
***E. coli* panel**[64]	x	x	x	Plate count[65]	<20 cfu/g[60]
***Campylobacter* species screen**	x	x		RT-PCR[66]	Non detect 25g[60]
Salmonella	x	x	x	RT-PCR[67]	Non detect 25g[60]
Listeria monocytogenes	x	x	x	RT-PCR[68]	Non detect 25g
Staphylococcus aureus	x	x	x	Plate count[69]	<20 cfu/g[70]
Bacillus cereus	x	x		Plate count[71]	<1,000 cfu/g[69]
***Clostridium perfringens* toxin**	x			PET-RPLA[72]	<2 ng/ml[73]
***Staphylococcus* enterotoxin**	x			ELFA[74]	Not detected
Arsenic	x			ICP-MS[75]	<50 ppb
Cadmium	x			ICP-MS[74]	<20 ppb
Mercury	x			ICP-MS[74]	<20 ppb
Lead	x			ICP-MS[74]	<50 ppb
Norovirus	x			RT-PCR[76]	Negative
Hepatitis A virus	x			RT-PCR[76]	Negative
Biogenic amines: histamine and tyramine	x			LC-MS[77]	<50 mg/kg[78]

All tests were conducted by accredited laboratories using methods validated for their intended purpose.

In order to address the potential production of heat-resistant microbial toxins, such as Staphylococcal enterotoxins[79] or Clostridial toxins[80] that may resist Wildtype's microbial inactivation (thermal kill) step,

[59] Association of Official Analytical Chemists (AOAC) 966.23 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[60] Gilbert RJ, de Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. Commun Dis Public Health. 2000 Sep;3(3):163-7. PMID: 11014026.
[61] FDA Bacteriological Analytical Manual, Chapter 18
[62] Compendium of Methods for the Microbiological Examination of Foods, Chapter 8 and 9.62 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[63] Compendium of Methods for the Microbiological Examination of Foods, Chapter 9.933. Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[64] Pathogenic (*Escherichia coli* O157:H7, *Escherichia coli* (Shiga toxin-producing)) and non-pathogenic
[65] Compendium of Methods for the Microbiological Examination of Foods, Chapter 9.933
[66] Association of Official Analytical Chemists (AOAC) RI #040702 (A) / AOAC-PTM 040702
[67] Association of Official Analytical Chemists (AOAC) RI 121501 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[68] Association of Official Analytical Chemists (AOAC) RI 061703 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[69] FDA Bacteriological Analytical Manual, Chapter 12 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[70] Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London:Health Protection Agency, November 2009.
[71] FDA Bacteriological Analytical Manual, Chapter 14 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[72] FDA Bacteriological Analytical Manual, Chapter 16
[73] Set at lower limit of detection for this assay
[74] Association of Official Analytical Chemists (AOAC) RI-ELFA
[75] Association of Official Analytical Chemists (AOAC) 993.14. Accreditation: ISO/IEC 17025:2017 A2LA 2918.01
[76] Eurofins Microbiology Laboratories internal testing methodology, 2022
[77] ISO 19343:2017 (E), modified
[78] Biji KB, Ravishankar CN, Venkateswarlu R, Mohan CO, Gopal TK. Biogenic amines in seafood: a review. J Food Sci Technol. 2016 May;53(5):2210-8. doi: 10.1007/s13197-016-2224-x. Wildtype acknowledges FDA's draft guidance lowering histamine levels to lower than 35 ppm. We will update this specification once that draft guidance is finalized.
[79] Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. Toxins (Basel). 2010 Aug;2(8):2177-97. doi: 10.3390/toxins2082177. Epub 2010 Aug 18. PMID: 22069679
[80] Uzal FA, Freedman JC, Shrestha A, Theoret JR, Garcia J, Awad MM, Adams V, Moore RJ, Rood JI, McClane BA. Towards an understanding of the role of Clostridium perfringens toxins in human and animal disease. Future Microbiol. 2014;9(3):361-77. doi: 10.2217/fmb.13.168. PMID: 24762309

finished product testing for both *Staphylococcus* enterotoxin and *Clostridium perfringens* toxin is performed as described above; results from three non-consecutive production batches are reported in Figure 6. The potential creation of biogenic amines during production (such as histamine and tyramine) is addressed in the response to question 16 below, and results of biogenic amine testing are reported in Figure 6.

16: Cell Growth in Bioreactor - Information Requested

On page 50 of the disclosable safety narrative, you state that decomposition of scombroid toxin-forming species is not relevant, but do not elaborate further or discuss scombroid toxin-forming species anywhere else in the disclosable safety narrative. High histamine levels in Atlantic salmon have been reported in the past.[81] Please describe why scombroid toxin-forming species are or are not relevant in your production process.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Scombroid toxicity in seafood is the result of bacteria-mediated spoilage, leading to the conversion of free amino acids to biogenic amines such as histamine and tyramine. A variety of bacteria are responsible for the generation of these biogenic amines; these are typically found within the skin, GI tract, and gills of fish (reviewed in Visciano et al.)[82] Given the described controls to preclude bacterial introduction and growth, the presence of a terminal lethal process, and testing to identify microbial contaminants (detailed in Figure 5), the primary causes of biogenic amine toxicity (resident bacteria) are excluded through process controls.

In order to verify this assumption and exclude the possibility that biogenic amines are created in the production process, the final product has also undergone repeated testing for two biogenic amines: histamine and tyramine, on account of their particular relevance to potential food toxicity[83] (results detailed in Figure 6).

17. Cell Harvest - Information Requested

On page 27 of the disclosable safety narrative, you state "Two potential hazards were identified at this stage: growth of pathogens during cell culture and metal fragments from metal-to-metal contact. Both of these potential hazards are discussed above" but do not state whether any preventative controls are

[81] For example, World Health Organization & Food and Agriculture Organization of the United Nations (2018). Histamine in salmonids. Accessible at: https://www.who.int/publications/i/item/9789241514439
[82] Visciano P, Schirone M, Paparella A. An Overview of Histamine and Other Biogenic Amines in Fish and Fish Products. Foods. 2020 Dec 3;9(12):1795. doi: 10.3390/foods9121795. PMID: 33287193; PMCID: PMC7761699.
[83] Biji KB, Ravishankar CN, Venkateswarlu R, Mohan CO, Gopal TK. Biogenic amines in seafood: a review. J Food Sci Technol. 2016 May;53(5):2210-8. doi: 10.1007/s13197-016-2224-x. Epub 2016 May 29. PMID: 27407186; PMCID: PMC4921096.

in place at this stage. Page 20, Figure 20 of the disclosable safety narrative, which lists the potential hazards identified at the cell harvest stage, includes two preventative controls. Please clarify, for addition to the disclosable safety narrative, this discrepancy.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture
Cell Maturation

Wildtype Response: The controls discussed on page 20 for this stage of the process are indeed in place. Further or duplicate discussion of these two controls was omitted on page 27 for brevity.

18. Cell Harvest - Information Requested

On pages 28 and 29 of the disclosable safety narrative, you state "The validation of this pathogenic eradication step occurs with routine testing of the final product for infectious and toxin-mediated pathogenic agents"; however, on page 21, Figure 20, which lists the potential hazards identified at the cell maturation stage, does not mention routine testing or toxins. Please clarify, for addition to the disclosable safety narrative, this statement and discuss how frequently "routine testing of the final product" occurs, as well as what type of analyses are performed (e.g., microorganisms, toxins) and their corresponding specifications.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Please refer to Figure 5 above for Wildtype's testing plan and specifications. Initial testing is designed to be comprehensive in nature to ensure Wildtype's preventive controls are functioning as intended. Once a baseline is established in a production environment, a risk-based approach will be employed to calibrate the frequency and specific slate of potential biological hazard testing.

19. Cell Harvest - Information Requested

On page 28 of the disclosable safety narrative, you describe the cell maturation stage. Please state, for addition to the disclosable safety narrative, how long this processing stage occurs (and at what temperature is the product held during maturation). Please also include how long the product is stored at 4°C, as described on page 29 of the disclosable safety narrative.

Significance

This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture.

Wildtype Response: Wildtype's first generation products have a limited cell maturation stage consisting of a process that lasts approximately one hour with a temperature range of 20-53˚C. The product is then subjected to a number of processing steps as well as a terminal lethal step that ensures internal temperature reaches at least 70˚C and remains at this temperature for at least 25 minutes. The product is then cooled in a refrigerator and stored at 4˚C for at least six hours before being stored for final shipment in a standard freezer (-20˚C). This process is followed by the comprehensive confirmatory screens for potential pathogens described in Figure 5 above until a baseline is established, after which point testing for pathogens will be conducted on a risk-adjusted basis. All steps from cell proliferation until final processing take place in a Current Good Manufacturing Practice (cGMP) compliant facility. Although the cell proliferation stages occur under strictly aseptic conditions, the limited cell maturation stage and subsequent terminal lethal steps occurs without aseptic controls in an environment-controlled, high-care food production facility, where hygienic zoning and extra gowning requirements are present.

20. Cell Harvest - Information Requested

On page 29 of the disclosable safety narrative, "… toxin-mediated pathogenic agents" are mentioned but are not described in detail. Please elaborate on, for addition to the disclosable safety narrative, what is meant by "toxin-mediated pathogenic agents".

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture.

Wildtype Response: The original text was written to distinguish the potential for infectious microbial pathogenesis (e.g. *Staphylococcus aureus* infection) from that of toxin-mediated pathogenesis (e.g. Staphylococcal enterotoxin). Both classes of adventitious agents are tested as detailed in Figure 5.

21. Cell Harvest - Information Requested

On page 29 of the disclosable safety narrative, you describe the validation experiments on the thermal step performed, and state, "A preliminary validation of this thermal step was completed in April 2022". Please describe whether further validation studies are planned.

Further, on page 29 of the disclosable safety narrative, you describe the validation experiments on the thermal step performed, and state, "Salmonella spp. were chosen for their resilience to heating"; however, you do not provide a reference for this statement, nor do you identify the species and serovar of Salmonella used for this study. As an example, there are reports in the literature of Listeria

monocytogenes as being thermotolerant,[84] however, several authors note that this depends on the strain, the food substrate, and other characteristics (e.g., sugar and salt levels, alkalinity, acidity).[85] Please elaborate on, for addition to the disclosable safety narrative, the statement on page 29, and provide a robust discussion with complete citations from relevant peer-reviewed literature.

Furthermore, please discuss whether the production of microbial toxins, including heat resistant toxins, is a safety concern in your production process, particularly during cell maturation.

Also, please clarify whether this process preventive control (i.e., "Product is heated to lethal temperature") is employed for all finished food products produced from the harvested cellular material, including the ready-to-eat (RTE) products such as salmon nigiri. If this process preventive control is not employed for RTE products, such as salmon nigiri, what preventive controls are employed to mitigate contamination by the biological hazards identified as risks during the cell maturation step (page 21)?

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, including the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response:

The completed validation study used a cocktail of five *Salmonella* serovars comprising S. typhimurium, *S.* enteritidis, *S.* seftenberg, *S.* infantis, and S. newport. These serovars were used to make 8-log cocktails. Pure cultures were introduced to Tryptic Soy Broth (TSB) and cultured overnight for 18-24 hours at 37°C, a 100 µL aliquot is recultured in TSB again overnight at 37°C for 18-24 hours. Each of the 5 strains were combined to make a single cocktail. The cocktail approach in Wildtype's validation study was selected to account for a range of *Salmonella* serovars. S. senftenberg was selected as an example of a particularly thermo-resistant strain. For example, an older study[86] compared 75 different serotypes of *Salmonella* and found none to be as heat-resistant as S. senftenberg. The strains were then introduced to Wildtype salmon in the validation study.

This validation study was informed by a recent thermal inactivation of *Salmonella* study conducted at Texas Tech University.[87] The authors used a similar cocktail of *Salmonella* strains, including the thermotolerant serotype Salmonella senftenberg. In ground beef trimmings with a heat treatment

[84] Doyle, M. E. et al. (2001). Heat resistance of Listeria monocytogenes. Journal of Food Protection, 64(3), p. 410-429. doi: 10.4315/0362-028x-64.3.410
[85] Doyle, M. E. and Mazzotta, A. S. (2000). Review of studies on the thermal resistance of Salmonellae. Journal of Food Protection, 63(6), p. 779-795. doi: 10.4315/0362-028x-63.6.779
[86] Ng H, Bayne HG, Garibaldi JA. Heat resistance of Salmonella: the uniqueness of Salmonella senftenberg 775W. Appl Microbiol. 1969 Jan;17(1):78-82. doi: 10.1128/am.17.1.78-82.1969. PMID: 5774764; PMCID: PMC377616.
[87] Ramirez-Hernandez A, Inestroza B, Parks A, Brashears MM, Sanchez-Plata MX, Echeverry A. Thermal Inactivation of Salmonella in High-Fat Rendering Meat Products. J Food Prot. 2018 Jan;81(1):54-58. doi: 10.4315/0362-028X.JFP-17-126. PMID: 29257727.

temperature ranging from 60 to 121˚C, D values[88] in thermal death curves ranged from 0.087 minutes to 2.175 minutes.

A similar 2017 study[89] inoculated teriyaki chicken breasts with five *Salmonella* strains and five *L. monocytogenes* strains. Similar to Ramirez-Hernandez et al., the product was immersed in a circulating water bath and cooked to temperatures ranging from 55 to 60˚C. D values for *Salmonella* in the chicken breast ranged from 47.7 minutes at 55˚C to 7.5 minutes at 60˚C. D values for *L. monocytogenes* ranged from 54.8 minutes at 55˚C to 10.39 minutes at 60˚C. In this case, marination rendered the pathogens more sensitive to the lethal effects of heat.

Similar results were obtained in a 2004 study[90] of *Salmonella* and *L. monocytogenes* in ground chicken thigh and leg meat, as well as skin. A cocktail of 6 *Salmonella* serovars (S. senftenberg, S. typhimurium, S. neidelberg, S. mission, S. montevideo, and S. california) and six isolates of *L. monocytogenes* were used. D values of *Salmonella* at 55 to 70˚C were 43.33 to 0.07 minutes in meat and 43.76 to 0.09 minutes in the skin. D values of *L. monocytogenes* at 55 to 70˚C were 38.94 to 0.04 in the meat and 34.05 to 0.05 minutes in the skin.

Salmonella serovars were used for these initial validation studies because of their relative thermoresistance, as noted above; however, further validation studies are planned to expand the scope of the previous study (e.g., to include other potential pathogens such as *Listeria monocytogenes*).

The production of microbial toxins (both in early production steps and the later cellular maturation step) is a concern addressed in the response to question 15 above.

The referenced thermal inactivation step is employed for all finished food products from the harvested cellular material, including ready-to-eat (RTE) products such as salmon nigiri.

Product Characterization

22. Contaminant Analysis - Information Requested

On page 16 of the disclosable safety narrative, Figure 11 presents interim analytical data for the RTE, harvested cellular material; however, specifications are not provided for any of the analyses presented in the figure, nor is a statement confirming that the methods used are validated for their intended purpose. Furthermore, the figure does not include specifications and results of analytical testing for the presence of adventitious agents (e.g., toxic heavy metal analyses, microorganisms).

[88] The D value is a measure of the heat resistance of a microorganism: minutes at a given temperature required to destroy 1 log cycle (90%) of the target microorganism.
[89] Dimitrios Karyotis, Panagiotis N. Skandamis, Vijay K. Juneja, Thermal inactivation of Listeria monocytogenes and Salmonella spp. in sous-vide processed marinated chicken breast, Food Research International, Volume 100, Part 1, 2017, Pages 894-898, ISSN 0963-9969, https://doi.org/10.1016/j.foodres.2017.07.078.
[90] R.Y. Murphy, T. Osaili, L.K. Duncan, J.A. Marcy, Thermal Inactivation of Salmonella and Listeria Monocytogenes in Ground Chicken Thigh/Leg Meat and Skin, Poultry Science, Volume 83, Issue 7, 2004, Pages 1218-1225, ISSN 0032-5791, https://doi.org/10.1093/ps/83.7.1218.

Figures 3 and 4 on pages 8 and 9 of the disclosable safety narrative respectively, include toxic heavy metal and microbial analyses performed on various forms of the harvested cellular material (e.g., cells alone, cells and scaffold, cell culture media).

Please clarify, for addition to the disclosable safety narrative, how Figures 3 and 4 are related to Figure 11, and provide the corresponding specifications (e.g., toxic heavy metals, microorganisms) for the harvested cellular material.

Are additional microbial specifications for the harvested cellular material anticipated beyond those presented in Figure 4 (e.g., aerobic plate count, yeast and mold, coliforms)? Are specifications for secondary metabolites (e.g., biogenic amines) or toxins (e.g., scombroid toxin) anticipated? If not, please discuss, for addition to the disclosable safety narrative, why these microorganisms and contaminants do not pose a safety concern in your production process.

Significance
Specifications are an important element of identity and provide assurance of a performance standard for the control of certain contaminant risks. Further, this information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Figures 3 and 4 in the disclosable safety narrative represented a subset of safety testing for the harvested cellular material, while Figure 11 provided an interim nutritional analysis. All tests were carried out by accredited 3rd party laboratories and all methods were validated for their intended purpose.

Specifications for safety analyses are included in Figure 6 below. All methods are validated for their intended purpose. Figure 6 below also describes additional microbial, secondary metabolite, and toxin specifications for the harvested cellular material beyond those presented in Figures 3 and 4 in the disclosable safety narrative.

23. Contaminant Analysis - Information Requested

On page 52 of the disclosable safety narrative, you state “As part of its quality control processes, Wildtype plans to implement routine sampling and testing of these assays for initial batches of Wildtype seafood products, and to screen finished products for the material inputs discussed in earlier sections of this analysis”. Please provide, for addition to the disclosable safety narrative, the results of the analyses of three batches (preferably non-consecutive) of the finished product, including the complete specification parameters.

Significance

Batch analysis provides corroborative information in support of identity as well as assurance that the harvested cellular material conforms to the stated specifications. Further, this information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Initial testing of the finished product was designed to encompass the detection of pathogenic bacteria (*Bacillus cereus*, *Campylobacter spp.*, *E. coli*, *Enterobacteriaceae*, *Listeria monocytogenes*, *Salmonella spp.*, and *Staphylococcus aureus*), viruses (norovirus and Hepatitis A virus), toxic metal contaminants (arsenic, cadmium, lead, and mercury), and toxins of microbial origin (i.e. Staphylococcal enterotoxins, Clostridial toxins, and biogenic amines such as histamine). While some of these potential contaminants are included because of their presence in conventional seafood (i.e. toxic metal contaminants [shown in Figure 6] and biogenic amines[91]), others (such as norovirus and coliforms) are included to evaluate the potential risk for human transmission of pathogenic agents during production.

Figure 6 below presents the results of three representative, non-consecutive batches of the finished product with specification parameters. Staphylococcal enterotoxins were tested at a separate qualified third-party laboratory using three non-consecutive batches of the finished product; for this reason, these batches are designated "Wildtype Batch 4, 5, and 6." All tests are validated for their intended purposes.

[91] Visciano P, Schirone M, Paparella A. An Overview of Histamine and Other Biogenic Amines in Fish and Fish Products. Foods. 2020 Dec 3;9(12):1795. doi: 10.3390/foods9121795. PMID: 33287193; PMCID: PMC7761699.

Figure 6 – Testing for adventitious agents

Test	Wildtype salmon batch 1	Wildtype salmon batch 2	Wildtype salmon batch 3	Conventional coho	Method	Specification
Aerobic bacteria	<10 cfu	<10 cfu	<10 cfu	**78,000 cfu/g**	Plate count	<1,000 cfu/g
Molds	<10 cfu	<10 cfu	<10 cfu	60 (est) cfu/g	Plate count	<100 cfu/g
Yeast	<10 cfu	<10 cfu	<10 cfu	**860 cfu/g**	Plate count	<100 cfu/g
Bacillus cereus	<10 cfu	<10 cfu	<10 cfu	Not tested	Plate count	<1,000 cfu/g
Campylobacter spp.	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not tested	RT-PCR	Not detected per 25g
Coliforms: general	<10 cfu	<10 cfu	<10 cfu	<10 cfu	Plate count	<100 cfu/g
Escherichia coli	<10 cfu	<10 cfu	<10 cfu	<10 cfu	Plate count	<20 cfu/g
***Escherichia coli* O157:H7**	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not detected per 25g	RT-PCR	Not detected per 25g
***Escherichia coli* (Shiga toxin-producing)**	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not detected per 25g	RT-PCR	Not detected per 25g
Enterobacteriaceae	<10 cfu	<10 cfu	<10 cfu	**1,600 (est) cfu/g**	Plate count	<100 cfu/g
Listeria spp.	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not detected per 25g	RT-PCR	Not detected per 25g
Listeria monocytogenes	Not detected per 25g	Not detected per 25g	Not detected per 25g	**Detected** per 25g	RT-PCR	Not detected per 25g
Salmonella spp.	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not detected per 25g	RT-PCR	Not detected per 25g
Staphylococcus aureus	<10 cfu	<10 cfu	<10 cfu	<10 cfu	Plate count	<20 cfu/g
Vibrio spp.	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not tested	RT-PCR	Not detected per 25g
Norovirus G1 and G2	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not tested	RT-PCR	Not detected per 25g
Hepatitis A virus	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not tested	RT-PCR	Not detected per 25g
Histamine (biogenic amine)	<2 mg/kg	<2 mg/kg	<2 mg/kg	Not tested	LC-MS	<50 mg/kg[92]
Tyramine (biogenic amine)	<0.4 mg/kg	<0.4 mg/kg	<0.4 mg/kg	Not tested	LC-MS	<50 mg/kg
Arsenic	<0.01 ppm	<0.01 ppm	<0.01 ppm	**0.366 ppm**	ICP-MS	<50 ppb
Cadmium	<0.005 ppm	<0.005 ppm	<0.005 ppm	<0.005 ppm	ICP-MS	<20 ppb
Lead	<0.005 ppm	<0.005 ppm	0.008 ppm	<0.005 ppm	ICP-MS	<50 ppb
Mercury	<0.005 ppm	<0.005 ppm	<0.005 ppm	**0.066 ppm**	ICP-MS	<20 ppb
***Clostridium perfringens* toxin**	<2ng / ml	< 2ng/ml	<2 ng/ml	Not tested	PET-RPLA	<2 ng/ml

Test	Wildtype salmon Batch 4	Wildtype salmon Batch 5	Wildtype salmon Batch 6	Conventional coho	Method	Specification
***Staphylococcus* enterotoxin**	Not detected	Not detected	Not detected	Not tested	ELFA	Not detected

References for all methods are provided in Figure 5

[92] Visciano, P.; Schirone, M.; Paparella, A. An Overview of Histamine and Other Biogenic Amines in Fish and Fish Products. *Foods* 2020, *9*, 1795. https://doi.org/10.3390/foods9121795

Points of Clarification

Substances Used During Cell Culture

24. Daily Intake Calculations - Information Requested

Figure 14 on page 32 of the disclosable safety narrative presents the results of the expected concentration of 27 substances in the harvested cellular material. We observed some irregularities in the calculations for the values presented in Figure 14 (e.g., "growth factor/hormone"). Please review the values presented in Figure 14, confirm whether they are accurate, and provide a sample calculation as to how these values were derived. If any are not accurate, please provide a revised copy of Figure 14, for addition to the disclosable safety narrative. If the values are accurate, please discuss the significance of this estimate in the context of your overall safety narrative.

Further, most of the calculations presented in Table 2.8 on page 19 of the confidential supplementary material make sense based on the indicated salmon intake; however, at least two of the presented exposures do not follow the same arithmetic as the other exposures presented in the table. Please review the values presented in Table 2.8 and confirm whether they are accurate. If any are not accurate, please provide a revised copy of Table 2.8 (marking as confidential as needed). If the values are accurate, please discuss the significance of this estimate in the context of your overall safety narrative.

Significance

The basis for your conclusion of safety regarding any residual presence of these substances is an important element of your disclosable safety narrative and further discussion provides useful additional context regarding your conclusion.

Wildtype Response: Figure 14 of CCC 000005 was constructed with calculations for additional dilutions that occur early in the production process. Figure 2 above eliminates these assumptions for simplicity, and lists items no longer used in Wildtype's production process (Figure 2a). Although simplifying the calculated dilution steps likely overestimates the actual concentrations of inputs found in the final product, all values remain orders of magnitude below the detectable limit and below toxicological significance in each case (Figures 2b-d). Sample calculations for three inputs are listed below, applying to both Figure 2 (in response to question 5) and Figure 7 here.

Page 31 of 46 removed.

Removed page contains trade secret and confidential commercial information exempt from disclosure under exemption 4 of the Freedom of Information Act.

Three representative calculations are shown below:

Phenol red (only used prior to cell proliferation / food production):
Dilution steps: 3-6, 8 referenced in CCC 000005
Initial concentration: 13.86 mg/L

Calculated final concentration: 13.86 mg/L x 1.2E-13 {step 3} x 0.0005 {step 4} x 0.5 {step 5} x 0.005 {step 6} x 1E-12 {step 8} = 2.1E-30 mg/L

The final concentration (2.1E-30 mg/L) is multiplied by the 90th percentile average intake of salmon (1.54 g/kg bw/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (1.54 g/kg bw/day) x (1Lg / 1000 g)= 3.23E-33 mg/kg bw/day.

To obtain the daily intake in mg/day, the final concentration (2.1E-30 mg/L) is multiplied by the 90th percentile average intake of salmon (109 g/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (109 g/day) x (1L g / 1000 g)= 2.29E-31 mg/day.

Insulin:
Dilution steps: 8 referenced in CCC 000005
Initial concentration: 10 mg/L

Calculated final concentration: 10 mg/L x 1E-12 {step 8} = 1.09E-11 mg/L

The final concentration (1E-11 mg/L) is multiplied by the 90th percentile average intake of salmon (1.54 g/kg bw/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (1.54 g/kg bw/day) x (1Lg / 1000 g)= 1.54E-14 mg/kg bw/day.

To obtain the daily intake in mg/day, the final concentration (1E-11 mg/L) is multiplied by the 90th percentile average intake of salmon (109 g/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (109 g/day) x (1L g / 1000 g)= 1.09E-12 mg/day.

L-taurine:
Dilution steps: 8 referenced in CCC 000005
Initial concentration: 2.17 mg/L

Calculated final concentration: 2.17 mg/L x 1E-12 {step 8} = 2.17E-12 mg/L

The final concentration (2.17E-12 mg/L) is multiplied by the 90th percentile average intake of salmon (1.54 g/kg bw/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (1.54 g/kg bw/day) x (1Lg / 1000 g) = 3.39E-15 mg/kg bw/day.

To obtain the daily intake in mg/day, the final concentration (2.17E-12 mg/L) is multiplied by the 90th percentile average intake of salmon (109 g/day) to obtain the 90th percentile estimated intake of the compound (2.1E-30 mg/L) x (109 g/day) x (1L g / 1000 g)= 2.4E-13 mg/day.

In order to confirm the calculations of Figure 7, the concentrations of two representative inputs were directly measured in the final product. The first is FGF2, which was undetectable in the final product (Figure 3). The second is dimethyl sulfoxide, (DMSO - only used prior to cell proliferation / food production), which was also undetectable in the final product (<250 ppm[94], detailed in the analytical report "DMSO" [report ID: AR-20-QR-017260-01], appended to this document). Both tests were validated for their intended purposes.

Cell Culture Process
Adventitious Agent Hazard Assessment
25. Adventitious Agent Hazard Assessment - Information Requested

On page 24 of the disclosable safety narrative, you describe processes used to sterilize equipment, including gamma irradiation. Please clarify, for addition to the disclosable safety narrative, whether irradiation is performed in-house.

Significance
This information will better help readers of the disclosable safety narrative to understand your cell culture process.

Wildtype Response: Gamma irradiation is not performed in-house. Consumable equipment (such as pipettes and cell culture vessels) is purchased from vendors that have subjected these supplies to gamma irradiation for the purpose of sterilization, according to accepted industry standards. When components are sterilized in-house, a steam autoclave that has been qualified is used.

26. Cell Harvest - Information Requested

Please provide, for addition to the disclosable safety narrative, the material presented in Section 2.5 (page 17) of the confidential supplementary material.

Significance
This information will better help readers of the disclosable safety narrative to understand your cell culture process.

Wildtype Response: No additional material inputs are used at this stage. Cells are stored in standard 4˚C refrigeration or frozen (-20 or -80˚C) prior to being seeded onto Wildtype scaffolds.

[94] Detection limit for this analytical test is 250 ppm. Chemical Abstracts Service (CAS) Registry # 67-68-5.

27. Control of Potential Allergens - Information Requested

On page 26 of the disclosable safety narrative, you state "… Wildtype works with several types of seafood, each with a unique allergen profile" in the facility and explain that labeling control is applied to ensure the correct seafood species is used. However, on page 50, you state "All of Wildtype's current production centers on only one species: coho salmon (*Oncorhynchus kisutch*). As such, the potential for allergen cross-contact during production is negligible". Please clarify, for addition to the disclosable safety narrative, whether an allergen program is developed and implemented within the production facility which covers all the processing steps (other than cell thawing), and whether any considerations are given for the potential for allergen contamination from any of the material inputs or other ingredients, as well as any other potential sources.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the processes in place to control for the presence of allergens.

Wildtype Response: Wildtype's production facility is focused only on one species: Coho salmon. Research and development is conducted in a separate facility, and cell lines for R&D are stored in separate, dedicated liquid nitrogen dewars.

Wildtype has implemented and maintains an allergen control and documentation program within its production facilities that encompasses all steps of the production process (including inputs used in the plant-based scaffold, for example). Potential allergens are annotated in the raw materials specification for each input or processing aid, and packaging for customers discloses all allergens present in the finished product.

Product Characterization
28. Intended Use - Information Requested

On page 15 of the disclosable safety narrative, you present a sample of the harvested cellular material used to produce salmon nigiri, and further present interim analytical data for the RTE, harvested cellular material in Figure 11 on page 16. For addition to the disclosable safety narrative, please clarify the intended use and preparation of the harvested cellular material (i.e., the finished food products); is the intended use limited to RTE products?
Significance
This information will better help FDA and readers of the disclosable safety narrative to understand the intended use of the harvested cellular material. Products that are RTE, versus those subjected to further downstream food processing steps, will have different food safety plans and hazard analysis and critical control points (HACCP) plans.

Wildtype Response: Wildtype's initial product, a salmon "saku" block (pictured below) is intended to be limited to ready-to-eat (RTE) products. Wildtype's food safety and HACCP plans are customized to the requirements of a RTE food product.

Figure 8 - Wildtype's initial product: "Wildtype salmon saku"

29. Contaminant Analysis - Information Requested

Figure 3 on page 8 of the disclosable safety narrative includes toxic heavy metal analyses performed on various forms of the harvested cellular material (e.g., cells alone, cells and scaffold, cell culture media) and conventional salmon. Please provide, for addition to the disclosable safety narrative, the limit of detection for each of the analyses performed. Please provide copies of the actual analytical results.

Significance

This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: The limits of detection for these tests are listed in Figure 9 below:

Figure 9 – Toxic heavy metal limits of detection

Compound	Limit of detection
Arsenic	10 ppb
Mercury	5 ppb
Cadmium	5 ppb
Lead	5 ppb

All methods are validated for their intended purpose. A copy of the referenced analytical results is appended to this document according to the following naming conventions:
Metals: Cells alone (report name "Cells for metals") report ID: AR-22-QR-009870-01
Metals: Cells and scaffold (report name "Saku for metals") report ID: AR-22-QR-010007-01
Metals: Cell culture media (report name "Media for metals") report ID: AR-22-QR-009871-01
Metals: Conventional salmon (report name "Wild King") report ID: AR-22-QR-012072-01

30. Contaminant Analysis - Information Requested

Figures 4 on page 9 of the disclosable safety narrative includes toxic heavy microbial analyses performed on various forms of the harvested cellular material (e.g., cells alone, cells and scaffold, cell culture media). The results of these analyses are compared to farmed Atlantic salmon. As your harvested cellular material is derived from Pacific salmon, please provide data from Pacific salmon, if available, or a discussion regarding why data from farmed Atlantic salmon may be extrapolated as a conventional comparator.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Wild coho Pacific salmon (*Oncorhynchus kisutch*) has replaced farmed Atlantic salmon as a conventional comparator in the table below:

Figure 10 – Testing of Coho salmon

Potential pathogen	Potential source of contamination	Result for cells alone	Result for cell culture media	Result for the finished product (cells + scaffold)	Result for conventional salmon (wild Coho)	Detection limit (LOD)	Method
Listeria monocytogenes	Environmental and human handling	Not detected per 25g	Not detected per 25g	Not detected per 25g	**Detected** per 25g	0 / 25g	RT-PCR
Salmonella spp.	Human handling	Not detected per 25g	< 10 cfu/g	< 10 cfu/g	Not detected per 25g	0 / 25g	RT-PCR
Staphylococcus aureus	Human handling	<10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu / g	10 cfu/g	Culture (non-chromogenic media)

All methods used are validated for the intended purpose. Method references are provided in Figure 5.

Appendix: analytical reports

Analytical report 1 – Metals: Cells alone (report name “Cells for metals”) report ID: AR-22-QR-009870-01

Analytical report 2 – Metals: Cells and scaffold (report name “Saku for metals”) report ID: AR-22-QR-010007-01

Analytical report 3 – Metals: Cell culture media (report name “Media for metals”) report ID: AR-22-QR-009871-01

Analytical report 4 – DMSO (report name “WT July prototype”) report ID: AR-20-QR-017260-01

Analytical report 5 – Conventional salmon (report name “Wild King”) report ID: AR-22-QR-012072-01

Analytical report 1 – Metals: Cells alone (report name "Cells for metals") report ID:

AR-22-QR-009870-01

Eurofins Microbiology Laboratories (Los Angeles)

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-009870-01

Received On: 22Mar2022
Reported On: 29Mar2022

Eurofins Sample Code:	111-2022-03220075	**Sample Registration Date:**	22Mar2022
Client Sample Code:	Cells for metals	**Condition Upon Receipt:**	acceptable, 1.9°C
Sample Description:	Cells for metals	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	Reference	Accreditation	Completed	Sub
	AOAC 2011.19 and 993.14 (modified)	ISO/IEC 17025:2017 A2LA 2918.01	29Mar2022	1

Parameter	Result
Arsenic	<0.0100 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

Subcontracting partners:
1 - Eurofins Food Chemistry Testing US Madison, WI

Respectfully Submitted,

Viridiana Castro
Assistant Laboratory Manager

Analytical report 2 – Metals: Cells and scaffold (report name "Saku for metals") report ID: AR-22-QR-010007-01

eurofins | **Eurofins Microbiology Laboratories (Los Angeles)**

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-010007-01

Received On: 22Mar2022
Reported On: 30Mar2022

Eurofins Sample Code:	111-2022-03220077	**Sample Registration Date:**	22Mar2022
Client Sample Code:	Saku for metals	**Condition Upon Receipt:**	acceptable, 1.9°C
Sample Description:	Saku for metals	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	Reference	Accreditation	Completed	Sub
	AOAC 2011.19 and 993.14 (modified)	ISO/IEC 17025:2017 A2LA 2918.01	30Mar2022	1

Parameter	Result
Arsenic	<0.0100 ppm
Cadmium	<0.00500 ppm
Lead	0.00741 ppm
Mercury	<0.00500 ppm

Subcontracting partners:
1 - Eurofins Food Chemistry Testing US Madison, WI

Respectfully Submitted,

Viridiana Castro
Assistant Laboratory Manager

Analytical report 3 – Metals: Cell culture media (report name "Media for metals") report ID: AR-22-QR-009871-01

eurofins | **Eurofins Microbiology Laboratories (Los Angeles)**

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-009871-01

Received On: 22Mar2022
Reported On: 29Mar2022

Eurofins Sample Code:	111-2022-03220080	**Sample Registration Date:**	22Mar2022
Client Sample Code:	Media for metals	**Condition Upon Receipt:**	acceptable, 1.9°C
Sample Description:	Media for metals	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	Reference	Accreditation	Completed	Sub
	AOAC 2011.19 and 993.14 (modified)	ISO/IEC 17025:2017 A2LA 2918.01	29Mar2022	1

Parameter	Result
Arsenic	<0.0100 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

Subcontracting partners:
1 - Eurofins Food Chemistry Testing US Madison, WI

Respectfully Submitted,

Viridiana Castro
Assistant Laboratory Manager

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-20-QR-017260-01

Received On: 10Jul2020
Reported On: 26Jul2020

Eurofins Sample Code:	111-2020-07100149	**Sample Registration Date:**	10Jul2020
Client Sample Code:	WT July prototype	**Condition Upon Receipt:**	acceptable, 1.7°C
Sample Description:		**Sample Reference:**	

FS033 - Residual Solvents - Class 2 Mix C, Class 3 DMSO (Nonvolatile Solvents)	**Reference**	**Completed**	**Sub**
	No Reference	15Jul2020	1

Parameter	**Result**
2-Ethoxyethanol	<160 ppm
2-Methoxyethanol	<50.0 ppm
Dimethyl Sulfoxide	<250 ppm
N,N-Dimethylacetamide	<1,090 ppm
Ethylene Glycol	<620 ppm
Formamide	<220 ppm
N,N-Dimethylformamide	<880 ppm
N-Methylpyrrolidone	<530 ppm
Sulfolane	<160 ppm

Report Comment:
Refer to 2932839-0_COA for parameter limits per USP <467>.

Subcontracting partners:
1 - Eurofins Food Chemistry Testing US Madison, WI

Respectfully Submitted,

Viridiana Castro
Microbiology Supervisor

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-20-QR-017260-01

Received On: 10Jul2020
Reported On: 26Jul2020

WILDTYPE

Analytical report 5 – Conventional salmon (report name "Wild King") report ID:

AR-22-QR-012072-01

eurofins | **Eurofins Microbiology Laboratories (Los Angeles)**

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-012072-01

Received On: 06Apr2022
Reported On: 14Apr2022

Eurofins Sample Code: 111-2022-04060134
Client Sample Code: Wild King
Sample Description: Wild King
Sample Registration Date: 06Apr2022
Condition Upon Receipt: acceptable, 1.4°C
Sample Reference:

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	Reference	Accreditation	Completed	Sub
	AOAC 2011.19 and 993.14 (modified)	ISO/IEC 17025:2017 A2LA 2918.01	14Apr2022	2

Parameter	Result
Arsenic	0.729 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	0.0357 ppm

FS033 - Residual Solvents - Class 2 Mix C, Class 3 DMSO (Nonvolatile Solvents)	Reference	Accreditation	Completed	Sub
	USP 467 modified	ISO/IEC 17025:2017 A2LA 2918.01	14Apr2022	2

Parameter	Result
Residual Solvents - Class 2 Mix C, Class 3 DMSO	See attached report "3635136-0"

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.	ISO/IEC 17025:2017 A2LA 2927.01	13Apr2022	1

Parameter	Result
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %
C12:0 (Lauric Acid)	<0.02 %
C14:0 (Myristic acid)	0.72 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	0.03 %

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-012072-01

Received On: 06Apr2022
Reported On: 14Apr2022

Eurofins Sample Code:	111-2022-04060134	**Sample Registration Date:**	06Apr2022
Client Sample Code:	Wild King	**Condition Upon Receipt:**	acceptable, 1.4°C
Sample Description:	Wild King	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 996.06 mod.	ISO/IEC 17025:2017 A2LA 2927.01	13Apr2022	1

Parameter	**Result**
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	1.58 %
C16:1 Omega 7	0.86 %
C16:1 Total (Palmitoleic Acid + isomers)	0.97 %
C16:2 (Hexadecadienoic Acid)	0.07 %
C16:3 (Hexadecatrienoic Acid)	0.03 %
C 16:4 (Hexadecatetraenoic Acid)	0.07 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	<0.02 %
C18:0 (Stearic Acid)	0.38 %
C18:1 (Vaccenic acid)	0.28 %
C18:1 Omega 9 (Oleic Acid)	2.15 %
C18:1, Total (Oleic Acid + isomers)	2.72 %
C18:2 Omega 6 (Linoleic Acid)	0.13 %
C18:2, Total (Linoleic Acid + isomers)	0.20 %
C18:3 Omega 3 (Alpha Linolenic Acid)	0.08 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	0.08 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	0.27 %
C18:4 Total (Octadecatetraenoic Acid)	0.27 %
C20:0 (Arachidic Acid)	<0.02 %
C20:1 Omega 9 (Gondoic Acid)	0.37 %
C20:1 Total (Gondoic Acid + isomers)	1.35 %
C20:2 Omega 6	0.02 %
C20:2 Total (Eicosadienoic Acid)	0.02 %
C20:3 Omega 3	<0.02 %
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %
C20:4 Omega 3	0.14 %
C20:4 Omega 6 (Arachidonic Acid)	0.03 %
C20:4, Total (Eicosatetraenoic Acid)	0.18 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.72 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	0.04 %
C22:0 (Behenic Acid)	<0.02 %
C22:1 Omega 9 (Erucic Acid)	0.06 %

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-012072-01

Received On: 06Apr2022
Reported On: 14Apr2022

Eurofins Sample Code:	111-2022-04060134	**Sample Registration Date:**	06Apr2022
Client Sample Code:	Wild King	**Condition Upon Receipt:**	acceptable, 1.4°C
Sample Description:	Wild King	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 996.06 mod.	ISO/IEC 17025:2017 A2LA 2927.01	13Apr2022	1

Parameter	**Result**
C22:1 Total (Erucic Acid + isomers)	0.93 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	0.16 %
C22:5 Docosapentaenoic Omega 6	<0.02 %
C22:5 Total (Docosapentaenoic Acid)	0.16 %
C22:6 Docosahexaenoic Omega 3	0.63 %
C24:0 (Lignoceric Acid)	<0.02 %
C24:1 Omega 9 (Nervonic Acid)	0.08 %
C24:1 Total (Nervonic Acid + isomers)	0.09 %
Total Omega 3 Isomers	2.04 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	0.23 %
Total Omega 7 Isomers	1.14 %
Total Omega 9 Isomers	2.68 %
Total Monounsaturated Fatty Acids	5.84 %
Total Polyunsaturated Fatty Acids	2.49 %
Total Saturated Fatty Acids	2.75 %
Total Trans Fatty Acids	0.27 %
Total Fat as Triglycerides	11.86 %
Total Fatty Acids	11.35 %

Subcontracting partners:

1 - Nutrition Analysis Center, IA
2 - Eurofins Food Chemistry Testing US Madison, WI

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-012072-01

Received On: 06Apr2022
Reported On: 14Apr2022

Respectfully Submitted,

Anne Chi
Business Unit Manager

Received: 20 April 2023
Responded: 3 May 2023

Overview

This document responds to the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 20 April 2023. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests

Source Cell Procurement and Health Assessment

1: Identity - Information Requested

On page 5 of the disclosable safety narrative, you state "Wildtype has developed its salmon cell lines from egg, alevin, and fry stages of development," and provide a health certificate for salmon eggs from a Canadian institute, The BC Centre for Aquatic Health Sciences Society (Figure 2), further stating that "… all fish being imported into the United States from Canada are subject to a health assessment by a Canadian Fish and Wildlife inspector, validating that the fish are healthy and free of infectious disease." On page 2 of the January 17, 2023, amendment, you state, "The salmon cell line described in CCC 000005 was derived solely from Coho salmon fry originating from a Washington state hatchery," and further state that "… muscle and connective tissue (containing myoblasts and fibroblasts, respectively) are removed from the donor fish." For addition to the disclosable safety narrative, please clarify:

- the relationship of Figure 2, as presented in the disclosable safety narrative, with the response provided on page 2 of the January 17, 2023, amendment; and
- whether the donor fish had health certificates or laboratory analyses to validate that the fish are healthy and free of infectious disease.

Significance

It is important to clarify any information provided regarding the health of the source cell species as it is an element of the hazard analysis.

Wildtype Response: At the time CCC 000005 was drafted, Wildtype was culturing cell lines from both eggs obtained from a Canadian hatchery as well as eggs and fry from a Washington state hatchery. Figure 2 in CCC 000005 was provided as an example of a health certificate provided to Wildtype for eggs obtained from Canada. Wildtype's coho salmon cell line currently used to create finished food products come entirely from healthy fry-stage salmon sourced from a Washington state hatchery. While the hatchery does not provide health certificates for individual fish, they periodically inspect representative samples of fish for signs of abnormal development, injury, parasites, spores, and pathogenic bacteria. PCR, histopathology, and necropsy are used in the inspections. Fish displaying any abnormalities were not provided to Wildtype. As discussed extensively in CCC 000005 and our amendment on January 17, 2023, contamination of a culture with an adventitious agent would be

readily apparent, and any risk of potential adventitious agents from a donor fish persisting through the manufacturing process into Wildtype's finished food products is mitigated by our extensive preventive controls and good manufacturing practices (GMPs).

Cell Line Establishment

2: Adventitious Agent Hazard Assessment - Information Requested

On page 2 of the January 17, 2023, amendment, you state "The salmon cell line described in CCC 000005 was derived solely from Coho salmon fry originating from a Washington state hatchery." On page 6 of the disclosable safety narrative, you state "Clostridium botulinum is frequently found in aquatic sediment." Clostridium botulinum has been shown to be present on trout and salmon fingerlings raised in freshwater ponds. Therefore, for addition to the disclosable safety narrative, please describe whether C. botulinum is anticipated to be a food safety risk in your production process and describe how the presence of C. botulinum is controlled for.

Significance

This information will better help readers of the disclosable safety narrative to understand your cell culture process, including the adventitious agent hazard assessment performed.

Wildtype Response: As described in CCC 000005, donor fish are first treated with hydrogen peroxide prior to beginning the cell line establishment process. Hydrogen peroxide has been shown in the literature[1,2,3] to effectively neutralize *C. botulinum* bacteria and spores. As an obligate anaerobe, *C. botulinum* germination from putative spores would be exceedingly unlikely in salmon cell culture, which occurs under invariably aerobic conditions. As a result, the potential presence of spores would be mitigated by successive washing and dilution with each cell passage. As context for the cell line currently used to create finished food products (described in CCC 000005), this process has occurred hundreds of times as the cell line has been kept in continuous culture since 25 December, 2018. Even if possible, the germination of remaining *Clostridium botulinum* spores would result in a cytotoxic contamination of the cultures that would be detected via routine pH monitoring and microscopy; these cultures would subsequently be terminated. GMP production practices, including the sterilization and cleaning of culture vessels, further mitigate the introduction of *Clostridium botulinum* during production.

Cell Culture Process

3: Hazard Analysis & Preventive Controls - Information Requested

On page 26 of the January 17, 2023, amendment, you state "... further validation studies are planned to expand the scope of the previous study (e.g., to include other potential pathogens such as Listeria

[1] McDonnell G. Zhu PC. Peroxygens and other forms of oxygen: their use for effective cleaning, disinfection and sterilization, *New Biocides Development: The Combined Approach of Chemistry and Microbiology, ACS Symposium Series*, 2006 New York, Oxford University Press (pg. 292-308)

[2] Finnegan M, Linley E, Denyer SP, et al. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms, *J Antimicrob Chemother*, 2010, vol. 65 (pg. 2108-15)10.1093/jac/dkq308

[3] Johnston MD, Lawson S, Otter JA. Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with Clostridium botulinum spores. J Microbiol Methods. 2005 Mar;60(3):403-11. doi: 10.1016/j.mimet.2004.10.021. PMID: 15649542.

monocytogenes)." For addition to the disclosable safety narrative, please confirm whether further validation studies have been completed and provide a summary of the study design(s), the parameters, and the results obtained. Further, please explain how the thermal processing step could adequately control for the presence of all biological hazards identified in your food safety plan.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, such as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: A confirmatory set of validation studies were completed at Texas Tech University by graduate researcher Sam Peabody under supervision of Dr. Marcos Sanchez-Plata in April 2023, illustrating that Wildtype's current thermal processing steps are also effective at eliminating *Listeria monocytogenes*.

Validation study design

Five strains of *L. monocytogenes* (19118, N1-023, N1-031, N1-054, and Scott A) were used to make 8-log cocktails. The Scott A. strain is established in the literature[4] as a heat-resistant strain of *L. monocytogenes*. Pure cultures were introduced to tryptic soy broth (TSB) and cultured overnight for 18-24 hours at 37°C. A 100 μL aliquot was recultured again in TSB overnight at 37°C for 18-24 hours. Each respective strain was combined to make a single cocktail. Prior to inoculation, the cocktail was sampled and enumerated using serial dilutions, microdilutions, and plated using Tryptic Soy Agar with the drop-plate method and spread plating.

Samples of Wildtype salmon were inoculated with a 250 μL aliquot of inoculum and massaged thoroughly. Samples were then vacuum sealed and kept at room temperature for 30 minutes, permitting *L. monocytogenes* attachment to the food matrix. Samples were then submerged in a circulating bath, filled with water at temperatures corresponding to Wildtype's thermal processing step. Bags were removed from the water bath at intervals according to the time allowed per thermal profiles. Upon removal, sample bags were immediately immersed in an ice bath water to halt the thermal treatment.

Sample bags were sanitized and aseptically opened. 25 mL BPW (Buffered Peptone Water) was added to the sample bag for stomaching at 230 RPM for 30 seconds. Aliquots were removed from the sample bag and serially diluted and plated onto Tryptic Soy Agar plates using microdilution and drop plating methods.

D values were calculated using R in linear regression on the negative inverse slope of the log CFU/g over time. Time point 0 data were used to establish the recovery limits. Five minutes after submergence was

[4] M.Ellin Doyle, Alejandro S. Mazzotta, Tim Wang, Dana W. Wiseman, Virginia N. Scott, Heat Resistance of Listeria monocytogenes, Journal of Food Protection, Volume 64, Issue 3, 2001, Pages 410-429, ISSN 0362-028X, https://doi.org/10.4315/0362-028X-64.3.410.

considered the beginning of the curve, as the initial five minutes are needed to ramp up to the target temperature. The graphic representation was modeled in R.

D value times are for temperatures/times below Wildtype's current operating limits in our production process.

Validation results

Table 1. Initial D-values for control of *L. monocytogenes* in Cell-Based Salmon

Temperature	D value (min/log(CFU))	Std Error
60°C	-60.0	45.0
65°C	12.4	1.60
70°C	1.94	0.275

These methods correspond to validated methods previously described in the literature.[5]

Applicability to other biological hazards

D values for *L. monocytogenes* are described above. D values for *Salmonella spp,* were described in CCC 000005. The third biological hazard of concern is *Staphylococcus aureus*, which has been shown in the literature[6] to demonstrate log reductions at temperatures lower than *Listeria* and *Salmonella.*

While the thermal processing step is an important component of Wildtype's preventive controls, we do not rely on it exclusively to mitigate all of the potential biological hazards described in our hazard analysis in CCC 000005. When CCC 000005 was drafted, Wildtype had not yet begun production operations and we anticipated potential hazards associated with our production process based on lab-scale experience. As of today's date, we have completed more than 90 days of pilot-scale GMP production, aimed at validating Wildtype's food safety plan and providing a microbiological "baseline" confirming that our preventive controls were effective. A summary of these data follows.

- Finished goods units produced: 57 in 9 lots on 9 production days
- Finished product testing within microbial specifications:[7] 100%
- Daily adenosine triphosphate (ATP) swabbing within specifications:[8] 100%
- Downstream processing area environmental monitoring program (EMP) swabs: 63
- Downstream EMP swabs within specification:[9] 100%

[5] Alejandra Ramirez-Hernandez, Brenda Inestroza, Amy Parks, Mindy M Brashears, Marcos X. Sanchez-Plata, Alejandro Echeverry; Thermal Inactivation of Salmonella in High-Fat Rendering Meat Products. J Food Prot 1 January 2018; 81 (1): 54–58.
[6] L Necidová, Š. Bursová, D. Haruštiaková, K. Bogdanovičová, I. Lačanin, Effect of heat treatment on activity of staphylococcal enterotoxins of type A, B, and C in milk, Journal of Dairy Science, Volume 102, Issue 5, 2019, Pages 3924-3932, ISSN 0022-0302, https://doi.org/10.3168/jds.2018-15255.
[7] For a list of adventitious agent testing and associated specifications, see Figure 5–"baseline" column in our response dated 17 January 2023 to FDA's questions.
[8] Specification for ATP: <10 RLU (Relative Light Unit) - this method is validated for its intended purpose and is carried out by an external laboratory (Aemtek)
[9] Specifications for all environmental monitoring at Wildtype are as follows: ATP: < 10 cfu/g, APC (aerobic plate count): < 100 cfu/g, Enterobacteriaceae: <10 cfu/g, yeast and mold: <100 cfu/g, *Listeria* spp: negative/not detected and *Salmonella* spp: negative/not detected. Water and air testing are also part of

- Upstream processing area EMP swabs: 33
- Upstream EMP swabs within specification: 100%
- Other processing area swabs: 63
- Other processing area swabs within specification: 98%

The data above show that Wildtype's food safety plan is working as intended and has successfully mitigated all potential biological and other hazards identified in the hazard analysis.

Product Characterization

4: Contaminant Analysis - Information Requested

Figure 5 on page 21 of the January 17, 2023, amendment includes toxic heavy metal and microbial analyses performed on the harvested cell material at various intervals (i.e., one-time, baseline, ongoing). On page 20 of the same amendment, you describe the frequency of "baseline" and "ongoing" analyses; however, you do not describe what "one-time" means in this context (e.g., one-time during each production run), and why some of the analyses presented in Figure 5 occur only "one-time." For each of the analyses you have identified as occurring "one-time" in Figure 5, please clarify if "one-time" refers to once per production run, or once overall. If it is the former, please identify the stage during production where testing occurs. Please also provide justification, for addition to the disclosable safety narrative, why these analyses occur only "one-time."

For example, on page 21 of the same amendment, you state, "In order to address the potential production of heat-resistant microbial toxins, such as Staphylococcal enterotoxins or Clostridial toxins that may resist Wildtype's microbial inactivation (thermal kill) step, finished product testing for both Staphylococcus enterotoxin and Clostridium perfringens toxin is performed as described above; results from three non-consecutive production batches are reported in Figure 6." Further, you list analysis for Staphylococcus enterotoxin as occurring "one-time," despite identifying Staphylococcus aureus as a microorganism of concern in your production process (page 9 of the disclosable safety narrative). For addition to the disclosable safety narrative, please clarify what specific controls are designed for eliminating Staphylococcus enterotoxin and what corrective action would be implemented if the resulting analysis was greater than the set specification.

Significance

Specifications are an important element of identity and provide assurance of a performance standard for the control of certain contaminant risks. It is important for FDA and readers of the disclosable safety narrative understand when analysis of adventitious agents occurs and how frequently it occurs during production. A rationale and discussion of the functional role for analyses that are performed "one-time" is important in understanding the appropriate controls you have in place to mitigate the presence of

Wildtype EMP program. Water is tested for coliforms - specification: <100 cfu / g. Air is tested for APC, yeast, and mold per the specifications above. All methods are validated for their intended purposes and are carried out by an external laboratory (Aemtek).

these adventitious agents. This information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: The "one-time" frequency described in Figure 5 of our January 2023 amendment is indeed one-time (testing was carried out for three non-consecutive batches in December 2022) and was used to illustrate the absence of a wide range of potential adulterants in our production process. Our hazard analysis did not identify the following potential adventitious agents as likely hazards in our production process (other than *Staphylococcus aureus*); however, testing data was provided in response to FDA questions about each of these potential adulterants in the November 2022 question set. The one-time tests were intended to demonstrate the absence of these theoretical hazards:

- *Clostridium perfringens* toxin
- *Staphylococcus* enterotoxin
- Arsenic
- Cadmium
- Mercury
- Lead
- Norovirus
- Hepatitis A virus
- Biogenic amines: histamine and tyramine

Staphylococcus enterotoxins are controlled in a similar manner to other conventional ready-to-eat foods, namely through the prevention of bacterial contamination by adhering to GMPs such as the use of gloves, gowns, hair nets, shoe covers/plant-dedicated shoes, and face masks in Wildtype's downstream processing areas. Food handlers are not permitted to work in the downstream processing area with any skin lesions.

During the 90-day "baseline" testing period, 9 lots of finished products were tested for *Staphylococcus aureus.* All samples were below the limit of detection (<10 cfu/g) and within the specifications as outlined in our January 27, 2023 amendment, Figure 5 (<20 cfu/g). The absence of *Staphylococcus aureus* in every lot indicates that Wildtype's general controls to mitigate against *Staphylococcus* growth and enterotoxin production are effective. *Staphylococcus aureus* testing is part of standard microbiological testing of every lot during this 90-day period. If the *Staphylococcus aureus* organism is not detected in the finished product, we can safely rule out the presence of any toxins produced by *Staphylococcal* organisms. In the event that *Staphylococcus* is detected in the finished product at levels greater than the specifications, the impacted product would be quarantined and Wildtype would follow standard corrective actions such as root cause analysis, added sanitation, enhanced environmental monitoring – all as outlined in our Food Safety, Recall (if applicable), and Sanitation plans.

5: Contaminant Analysis - Information Requested

Figure 5 on page 21 of the January 17, 2023, amendment includes toxic heavy metal and microbial analyses performed on the harvested cell material at various intervals (i.e., one-time, baseline, ongoing). On page 20 of the same amendment, you state, "These tests are performed on an ongoing basis (at least twice per year) based upon the results of our risk-based hazard analysis and preventative control plan as described in CCC 000005." Please clarify, for addition to the disclosable narrative, if the frequency of the "ongoing" analyses presented in Figure 5 occur "… at least twice per year." If so, please provide justification, for addition to the disclosable safety narrative, why the "ongoing" analyses only guarantees testing is performed twice per year, rather than for each batch of the harvested cell material.

Significance
Specifications are an important element of identity and provide assurance of a performance standard for the control of certain contaminant risks. This information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: The frequency of the "ongoing" analyses will occur at least twice per year, as correctly noted above. The risks of contamination by each of the 7 adventitious agents in question are effectively mitigated by Wildtype's production methods and risk mitigation strategies (including *L. monocytogenes*, as noted in the answer to question 3 above) as discussed in CCC 000005. For these reasons, the standard of biannual testing is included out of an abundance of caution; this is performed to ensure the efficacy of the company's hazard mitigation strategies over time. For context, it should be noted that 4 of these 7 adventitious agents are routinely detected in conventional salmon (reported in bold type within figure 6 of the January 17, 2023 amendment). As noted in our response to question 3 above, the adventitious agents subject to "ongoing" analysis were tested for and were not detected in all nine production lots during the 90 day "baseline" period; this confirms our risk-based assessment that these potential hazards either are not likely to occur because of the nature of our production process, or are effectively mitigated by our preventive controls.

6: Contaminant Analysis - Information Requested

On page 20 of the January 17, 2023, amendment you state "The potential generation of microbial toxins and biogenic amines is addressed by direct measurement of both classes of toxins." While Bacillus cereus is also briefly mentioned, and a "one-time" and "baseline" specification is set at <1000 CFU/g, you do not discuss the potential for sporulation of B. cereus (and other spore-forming microorganisms included on pages 20-21), nor the potential for the production of the B. cereus emetic toxin cereulide,

which is reported in the literature as being heat stable.[10] For addition to the disclosable safety narrative, please describe how you plan to control for the presence of B. cereus in the production process, including a discussion on mitigating the risk of B. cereus sporulation (and the sporulation of other spore-forming microorganisms you included on pages 20-21 of the January 17, 2023, amendment), as well as mitigating risks of toxin formulation in the final product.

Significance
This information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: As with other bacterial contaminants described above, Wildtype's strategy to mitigate *Bacillus cereus* sporulation and cereulide formation is centered around avoiding the introduction of this and other spore-forming microorganisms to the production process. This is largely achieved by adhering to GMPs such as the use of gloves, gowns, hair nets, shoe covers/plant-dedicated shoes, and face masks in Wildtype's downstream processing areas.

During the 90-day "baseline" testing period, 9 lots of finished products were tested for *Bacillus cereus, Salmonella,* and *Campylobacter.* All samples were within the specifications as outlined in our January 27, 2023 amendment for all 3 pathogenic bacteria. The absence of these bacteria in every lot indicates that Wildtype's general controls to mitigate bacterial contaminations are effective. *Bacillus cereus* testing is part of standard microbiological testing of every lot during the 90-day "baseline" period; testing for cereulide is obviated by consistent testing that reveals the absence of *Bacillus cereus* contamination, as the bacterium is required for toxin production. In the event that *Bacillus cereus* (or another spore-forming bacterium noted above) is detected in the finished product at levels greater than the specifications, the impacted product would be quarantined and Wildtype would follow standard corrective actions such as root cause analysis, added sanitation, enhanced environmental monitoring – all as outlined in our Food Safety, Recall (if applicable), and Sanitation plans.

Importantly, the toxicity of cereulide is likely to first present as cytotoxicity to salmon cells in culture, as it does in other *in vitro* cell culture systems.[11] *B. cereus* contamination would therefore likely be detected prior to testing, with impaired cell culture growth and production failures. Cell cultures displaying the presence of adventitious agents (described on page 19 of the January 17, 2023 amendment) are terminated. All stages of production from cell culture until final processing take place within a GMP compliant facility to further mitigate the risk of human pathogenic transmission. The product is stored at -20˚C for long term storage, further mitigating the risk for sporulation and toxin formation.

[10] Rajkovic, A., et al. (2008). Heat resistance of Bacillus cereus emetic toxin, cereulide, Letters in Applied Microbiology, 46(5), p. 536-541. doi: 10.1111/j.1472-765X.2008.02350.
[11] Andersson MA, Hakulinen P, Honkalampi-Hämäläinen U, Hoornstra D, Lhuguenot JC, Mäki-Paakkanen J, Savolainen M, Severin I, Stammati AL, Turco L, Weber A, von Wright A, Zucco F, Salkinoja-Salonen M. Toxicological profile of cereulide, the Bacillus cereus emetic toxin, in functional assays with human, animal and bacterial cells. *Toxicon*. 2007 Mar 1;49(3):351-67. doi: 10.1016/j.toxicon.2006.10.006. Epub 2006 Oct 27. PMID: 17156808.

7: Contaminant Analysis - Information Requested

Figure 6 on page 29 of the January 17, 2023, amendment lists the specifications for the harvested cell material and the results of three non-consecutive batch analyses. Your reported analytical data for arsenic, cadmium, lead, and mercury are substantially lower than your planned specification. If reasonably achievable, for addition to the disclosable safety narrative, please include revised specifications for arsenic, cadmium, lead, and mercury.

Significance
We encourage food manufacturers to adopt the lowest specifications for toxic heavy metals, particularly lead, that are reasonably achievable given their production process. There are recognized safety concerns associated with the presence of heavy metals in food. All reasonably achievable steps to limit the presence of heavy metals in the food supply should be taken.

Wildtype Response: Wildtype is committed to establishing appropriately stringent specifications for toxic heavy metals. We will analyze levels of heavy metals at least twice in the next 12 months and will consider lowering our specifications accordingly. In the interim, we note that our current specifications are the same as, or in most cases, substantially lower than those set out in CCC 000001 and CCC 000002.

8: Contaminant Analysis - Information Requested

Figure 10 on page 36 of the January 17, 2023, amendment includes the results for the analysis of L. monocytogenes, Salmonella serovars, and S. aureus in the cells alone, the cell culture media, as well as for the finished product (cells and scaffold). The detection limit provided for Salmonella serovars is "0/25 g," however, the results provided for the analysis of the cell culture media and for the finished product (cells and scaffold) were reported as <10 CFU/g. Figure 10 notes that human handling is the potential source of contamination for this biological hazard. For addition to the disclosable safety narrative, please clarify why the provided batch analyses show detection of Salmonella serovars in both the cell culture media and the finished product (cells and scaffold) and why this value is outside the specification of not detected per 25 g (Figure 5 (page 21)). Further, please describe what controls would be implemented to prevent contamination from this biological hazard and the corrective actions and verification of implementation and effectiveness.

Significance
This information will better help readers of the disclosable safety narrative to understand your cell culture process, including the parameters monitored during cell culture, as well as the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: Confirmation of results contained within the original analytical report for this data series revealed that a clerical error resulted in incorrect reporting for *Salmonella spp.* testing. The results

in question were reported as "< 10 cfu/g" in Figure 10 of the January 17, 2023 amendment, whereas the actual analytical report results were: "Not detected per 25g." The entire table has been revised to reflect this correction and is replicated below:

Potential pathogen	Potential source of contamination	Result for cells alone	Result for cell culture media	Result for the finished product (cells + scaffold)	Result for conventional salmon (wild Coho)	Detection limit (LOD)	Method
Listeria monocytogenes	Environmental and human handling	Not detected per 25g	Not detected per 25g	Not detected per 25g	**Detected** per 25g	0 / 25g	RT-PCR
Salmonella spp.	Human handling	Not detected per 25g	Not detected per 25g	Not detected per 25g	Not detected per 25g	0 / 25g	RT-PCR
Staphylococcus aureus	Human handling	<10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu / g	10 cfu/g	Culture (non-chromogenic media)

All methods used are validated for the intended purpose. Method references are provided in **Figure 5 - revised** at the end of this document.

The prevention of contamination from these biological hazards, corrective actions, and verification of implementation and effectiveness are addressed in response to question 14 (page 19) of the January 17, 2023 amendment and in the original disclosable safety narrative.

Testing of Wildtype's products has not resulted in the detection of these pathogens to date.

Points of Clarification

Source Cell Procurement and Health Assessment
9: Identity - Information Requested

On page 5 of the disclosable safety narrative, you state "Wildtype has developed its salmon cell lines from egg, alevin, and fry stages of development." On page 2 of the January 17, 2023, amendment, you state, "The salmon cell line described in CCC 000005 was derived solely from Coho salmon fry originating from a Washington state hatchery," and further state that "… muscle and connective tissue (containing myoblasts and fibroblasts, respectively) are removed from the donor fish … Potential biological hazards relevant to cells isolated from both eggs and juvenile fish originating from hatcheries include …" For addition to the disclosable safety narrative, please clarify whether only muscle and connective tissue (containing myoblasts and fibroblasts, respectively) are removed from the donor fish, or if eggs were also removed as well. Please provide detailed information about egg isolation and the applications, if applicable.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the source cell procurement.

Wildtype Response: The salmon cell line described in CCC 000005 and used for production purposes was exclusively derived from the muscle and connective tissue of coho salmon fry; eggs were not harvested or used at the time of the described cell isolation steps. Eggs were also not involved with subsequent cell line development, cell propagation, or production.

Substances Used During Cell Culture

10: Scaffold Production - Information Requested

On page 54 of the disclosable safety narrative, you state "Substances used in scaffolds are widely used in food production today in a manner consistent with Wildtype's use, and are used consistent with applicable regulatory requirements," and also provided additional data to support the safety of (2) inputs. On page 15 of the January 17, 2023, amendment, you state "Wildtype's scaffold formulation has been updated since CCC 000005 was submitted in June 2022." On page 16 of the same amendment, you provide a summary of the eight classes of substances used in the production of the scaffolds. For addition to the disclosable safety narrative, please provide a statement affirming (as well as corroborating justification) that all new scaffold inputs are safe at the levels found in the final product.

Significance

This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including characterization of the final product.

Wildtype Response: As with each of the disclosed product inputs, Wildtype affirms that all new scaffold inputs are safe at the levels and for the functions found in the final product. For any new scaffold input, Wildtype reviews existing regulatory requirements in terms of use and levels in the finished product to ensure the safety of those inputs. As previously stated, all scaffold inputs are widely used in the US food supply today in a manner consistent with Wildtype's use.

Cell Culture Process

11: Hazard Analysis & Preventive Controls - Information Requested

On page 25 of the January 17, 2023, amendment, you state "The completed validation study used a cocktail of five Salmonella serovars comprising S. Typhimurium, S. Enteritidis, S. Senftenberg, S. Infantis, and S. Newport … S. Senftenberg was selected as an example of a particularly thermo-resistant strain. For example, an older study[12] compared 75 different serotypes of Salmonella and found none to be as heat-resistant as S. Senftenberg." For addition to the disclosable safety narrative, please provide the strain names of the five serovars of Salmonella used in the completed validation study. Further, please clarify if the strain of S. Senftenberg used in your completed validation study is the same strain as cited in your response on page 25.

Significance

[12] Ng, H., Bayne, H.G., and Garibaldi, J.A. (1969). Heat resistance of Salmonella: the uniqueness of Salmonella senftenberg 775W. Applied Microbiology, 17(1), p. 78-82. doi: 10.1128/am.17.1.78-82.1969

This information will help FDA more clearly convey the identity of the product described in CCC 000005 at harvest when documenting our evaluation of your submission.

Wildtype Response: The five strains of *Salmonella* used in the validation study follow:

1. S. typhimurium: ATCC 14028 (Serotype: I 4,5,12:i:1,2)
2. S. enteritidis: ATCC 13076 No serotype listed, may have same designation as ATCC 25928 (Serotype: I 1,9,12:g,m).
3. S. newport: ATCC 6962 (Serotype I 6,8:e,h:1,2)
4. S. infantis: Note: this is a wildtype strain isolated from a Chilean meat processor confirmed by whole genome sequencing.
5. S. senftenberg: ATCC 43845, (Serotype: 1,3,19:g,s,t)

We confirm that the S. senftenberg strain was the same as used in the referenced paper (Ng et al). ATCC's product information sheet and peer reviewed literature[13] confirm that ATCC 43845 is the same strain as 775/W as referenced in Ng et al referenced below and our response on page 25.

12: Control of Potential Allergens - Information Requested

For addition to the disclosable safety narrative, please confirm if your allergen control program includes all other food allergens identified in the Food Allergen Labeling and Consumer Protection Act (FALCPA), as well as sesame which was added as a ninth allergen by the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the processes in place to control for the presence of allergens.

Wildtype Response: Wildtype's allergen control program includes consideration of all food allergens identified in FALCPA and the FASTER Act, including sesame.

Product Characterization
13: Contaminant Analysis - Information Requested

Figure 5 on page 21 of the January 17, 2023, amendment includes toxic heavy metal and microbial analyses performed on the harvested cell material at various intervals (i.e., one-time, baseline, ongoing). We were not able to locate footnote 74, based on the information provided. For addition to the disclosable safety narrative, please provide the complete citation for footnote 74.

[13] Nguyen SV, Harhay GP, Bono JL, Smith TPL, Harhay DM. 2017. Genome sequence of the thermotolerant foodborne pathogen Salmonella enterica serovar Senftenberg ATCC 43845 and phylogenetic analysis of loci encoding increased protein quality control mechanisms. mSystems 2:e00190-16. https://doi.org/10.1128/mSystems.00190-16

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand the analytical methods used for the harvested cell material specifications.

Wildtype Response: Footnote 74 of the January 17, 2023 amendment was correctly referenced for *Staphylococcus* enterotoxin testing by enzyme-linked fluorescent assay (ELFA) with AOAC R1-ELFA; a validation study for this method is found here:

Bennett RW. Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay-based methodology. *J Food Prot.* 2005 Jun;68(6):1264-70. doi: 10.4315/0362-028x-68.6.1264. PMID: 15954720.

In the same table, the measurement of cadmium, mercury, and lead incorrectly referenced the same citation, and should instead reference footnote 75. These three references are corrected in **Figure 5 – revised** at the end of this document.

14: Contaminant Analysis - Information Requested

Figure 5 on page 21 of the January 17, 2023, amendment lists the specification for the Escherichia coli panel (identified as E. coli O157:H7, Shiga toxin-producing E. coli, and non-pathogenic E. coli) as <20 CFU/g. Figure 6 on page 29 of the same amendment lists the specification for E. coli O157:H7, Shiga toxin-producing E. coli, and non-pathogenic E. coli as not detected per 25 g, not detected per 25 g, and <20 CFU/g, respectively.

The specifications for norovirus and hepatitis A virus, as presented in Figure 5 are listed as “negative”; however, in Figure 6 are listed as not detected per 25 g.

Figure 5 does not include specifications or frequency of analyses for Listeria spp. (but does include L. monocytogenes) or Vibrio spp.; however, specifications for both genera appear in Figure 6.
For addition to the disclosable safety narrative, please clarify these discrepancies by providing the accurate specification for each microbial specification listed above.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand the analytical methods used for the harvested cell material specifications.

Wildtype Response: These analytical methods and specifications are reconciled in the following table:

Test	Method	Specification
E. coli panel (non-pathogenic and pathogenic)	Plate count	<20 cfu/g
E. coli O157:H7	RT-PCR	Not detected per 25g
E. coli (Shiga toxin-producing)	RT-PCR	Not detected per 25g
L. monocytogenes	RT-PCR	Not detected per 25g
Listeria spp.	RT-PCR	Not detected per 25g
Vibrio spp.	RT-PCR	Not detected per 25g
Norovirus	RT-PCR	Not detected per 25g
Hepatitis A virus	RT-PCR	Not detected per 25g

The *E. coli* panel is a plate count test to identify all *E. coli* colony-forming units; it does not further assess bacterial genotypes to identify particular strains (such as *E. coli* O157:H7 or Shiga toxin-producing *E. coli*). RT-PCR is used to identify *E. coli* O157:H7 and Shiga toxin-producing *E. coli*, as shown above. *Listeria spp.* and *Vibrio spp.* testing was conducted for the purpose of comprehensive screening, but these tests are not part of Wildtype's finished product testing plan (detailed in **Figure 5 - revised** at the end of this document); *Listeria monocytogenes* testing replaces *Listeria spp.* testing for finished product testing and *Vibrio spp.* testing is not routinely performed, given the unlikely nature of its presence in the finished product (discussed on pages 6 and 7 of the disclosable safety narrative). Example results of each of these tests, with references and accreditations, are shown in the analytical report presented in response to question 17 below.

Norovirus and Hepatitis A virus are assessed by RT-PCR; the specification for both is set as "Not detected per 25g."

15: Contaminant Analysis - Information Requested

Figure 6 on page 29 of the January 17, 2023, amendment includes the results from the analysis of C. perfringens toxin. Please clarify whether the specification, <2ng/mL, is the limit of detection.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand the analytical methods used for the harvested cell material specifications.

Wildtype Response: As correctly noted above, the specification of <2 ng/ml is the limit of detection; all test results for *C. perfringens* toxin in figure 6 of the January 17, 2023 amendment were therefore interpreted to be not detected.

16: Contaminant Analysis - Information Requested

As presented in Figure 6 on page 29 of the January 17, 2023, amendment the results of the analysis of histamine (biogenic amine) and tyramine (biogenic amine) are listed as <2 mg/kg and <0.4 mg/kg respectively (across all three batches analyzed), while wild salmon is listed as not tested. The

specification for histamine and tyramine was set as 50 mg/kg. Please note that FDA issued a draft Compliance Policy Guide (CPG) that revises the current CPG Sec. 540.525 on decomposition and histamine in fish and fishery products on December 21, 2021.[14] If samples have 35 parts per million (ppm) or more histamine (lowered from 50 ppm), the FDA may consider the fish to be adulterated because they are decomposed and/or produced under insanitary conditions. Please take this into consideration when designing the specification for histamine.

Wildtype Response: Wildtype has noted the revised guidance set forth by the draft Compliance Policy Guide[15] that updates CPG Sec. 540.525, and the company has similarly updated its specification to < 35 parts per million (ppm), which is updated in **Figure 5 – revised** at the end of this document. As noted in the January 17, 2023 amendment, no production samples have demonstrated levels of histamine above or near this lower limit.

17. Contaminant Analysis - Information Requested

Analytical report 5 of the January 17, 2023, amendment (page 43) includes the certificates of analyses for conventional Wild King salmon (not Coho salmon, but Chinook salmon). As your harvested cellular material is derived from Coho salmon, please provide a discussion for why data from Wild King salmon is relevant and appropriate as a conventional comparator.

Significance
This information will better help FDA and readers of the disclosable safety narrative to understand your cell culture process, including the controls established to mitigate contamination by adventitious agents or other contaminants.

Wildtype Response: The described report (analytical report 5 of the January 17, 2023 amendment) addressed a request by FDA to provide the complete analytical reports of Wildtype's pre-market consultation submission, which included wild king (chinook) salmon as a conventional comparator. We agree that a more meaningful comparator is wild coho salmon; the analytical report corresponding to data reported in figure 6 of the January 17, 2023 amendment is attached below:

[14] FDA (2022). FDA Issues Draft Compliance Policy Guide for Decomposition and Histamine in Scombrotoxin (Histamine)-forming Fish and Fishery Products. Accessible at: https://www.fda.gov/food/cfsan-constituent-updates/fda-issues-draft-compliance-policy-guide-decomposition-and-histamine-scombrotoxin-histamine-forming
[15] Sec. 540.525 Scombrotoxin (Histamine)-forming Fish and Fishery Products – Decomposition and Histamine (CPG 7108.24) Draft Compliance Policy Guide Guidance for FDA Staff (linked here)

WILDTYPE

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Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-038311-01

Received On: 22Nov2022
Reported On: 01Dec2022

Eurofins Sample Code:	111-2022-11220022	**Sample Registration Date:**	22Nov2022
Client Sample Code:	Coho1	**Condition Upon Receipt:**	acceptable, 1.2°C
Sample Description:	Coho sample for FDA safety revision	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	**Reference** AOAC 2011.19, 993.14 and 2015.01 (modified)	**Accreditation**	**Completed** 01Dec2022	**Sub** 2

Parameter	**Result**
Arsenic	0.366 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	0.0666 ppm

UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 27Nov2022

Parameter	**Result**
Yeast	860 cfu/g

Parameter	**Result**
Moulds	60 (est) cfu/g

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 23Nov2022

Parameter	**Result**
Escherichia Coli O157:H7	Not Detected per 25 g

UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 24Nov2022

Parameter	**Result**
Staphylococcus aureus	< 10 cfu/g

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-038311-01

Received On: 22Nov2022
Reported On: 01Dec2022

Eurofins Sample Code:	111-2022-11220022	**Sample Registration Date:**	22Nov2022
Client Sample Code:	Coho1	**Condition Upon Receipt:**	acceptable, 1.2°C
Sample Description:	Coho sample for FDA safety revision	**Sample Reference:**	

UMJN3 - Non-O157 Shiga toxin-Producing Escherichia coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 23Nov2022	
Parameter	**Result**			
Non-O157 Shiga toxin-Producing Escherichia coli	Not Detected per 25 g			
UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 23Nov2022	
Parameter	**Result**			
Enterobacteriaceae	1,600 (est) cfu/g			
UMQDX - Listeria species - AOAC-RI 061702	**Reference** AOAC-RI 061702	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 24Nov2022	
Parameter	**Result**			
Listeria Species	Not Detected per 25 g			
UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 24Nov2022	
Parameter	**Result**			
Salmonella	Not Detected per 25 g			
UMQUI - Confirmation Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933		**Completed** 25Nov2022	
Parameter	**Result**			
Coliforms	< 10 cfu/g			
Parameter	**Result**			
Escherichia coli	< 10 cfu/g			
UMSAT - Vibrio spp - AOAC RI #050902	**Reference** AOAC-RI 050902	**Accreditation**	**Completed** 25Nov2022	**Sub** 1
Parameter	**Result**			
Vibrio spp	Not Detected per 25 g			
UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 24Nov2022	
Parameter	**Result**			

WILDTYPE

Wild Type, Inc.

Client Code: QR0000417

ANALYTICAL REPORT

AR-22-QR-038311-01

Received On: 22Nov2022
Reported On: 01Dec2022

Eurofins Sample Code:	111-2022-11220022	**Sample Registration Date:**	22Nov2022
Client Sample Code:	Coho1	**Condition Upon Receipt:**	acceptable, 1.2°C
Sample Description:	Coho sample for FDA safety revision	**Sample Reference:**	

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference**	**Accreditation**	**Completed**
	AOAC 966.23	ISO/IEC 17025:2017 A2LA 3329.05	24Nov2022

Parameter	**Result**
Aerobic Plate Count	78,000 cfu/g

UMXJM - Confirmation Listeria monocytogenes - AOAC-RI 061703	**Reference**	**Accreditation**	**Completed**
	FDA BAM Chapter 10	ISO/IEC 17025:2017 A2LA 3329.05	25Nov2022

Parameter	**Result**
Listeria monocytogenes	Detected per 25 g

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
2 - Eurofins Food Chemistry Testing US Madison, WI

Respectfully Submitted,

Viridiana Castro
Assistant Laboratory Manager

WILDTYPE

Figure 5 revised – Finished product testing

Potential Hazard	One time	Baseline	Ongoing	Method	Specification
Aerobic plate count	x	x	x	Plate count[59]	<1,000 cfu/g[60]
Yeast/mold	x	x	x	Plate count[61]	<100 cfu/g
Enterobacteriaceae	x	x	x	Plate count[62]	<100 cfu/g[60]
Coliforms	x	x		Plate count[63]	<100 cfu/g
***E. coli* panel**[64]	x	x	x	Plate count[65]	<20 cfu/g[60]
***Campylobacter* species screen**	x	x		RT-PCR[66]	Not detected per 25g[60]
Salmonella	x	x	x	RT-PCR[67]	Not detected per 25g[60]
Listeria monocytogenes	x	x	x	RT-PCR[68]	Non detect 25g
Staphylococcus aureus	x	x	x	Plate count[69]	<20 cfu/g[70]
Bacillus cereus	x	x		Plate count[71]	<1,000 cfu/g[69]
***Clostridium perfringens* toxin**	x			PET-RPLA[72]	<2 ng/ml[73]
***Staphylococcus* enterotoxin**	x			ELFA[74]	Not detected
Arsenic	x			ICP-MS[75]	<50 ppb
Cadmium	x			ICP-MS[75]	<20 ppb
Mercury	x			ICP-MS[75]	<20 ppb
Lead	x			ICP-MS[75]	<50 ppb
Norovirus	x			RT-PCR[76]	Not detected per 25g
Hepatitis A virus	x			RT-PCR[76]	Not detected per 25g
Biogenic amines: histamine and tyramine	x			LC-MS[77]	<35 mg/kg or ppm[78]

All tests were conducted by accredited laboratories using methods validated for their intended purpose.

[59] Association of Official Analytical Chemists (AOAC) 966.23 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[60] Gilbert RJ, de Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. Commun Dis Public Health. 2000 Sep;3(3):163-7. PMID: 11014026.
[61] FDA Bacteriological Analytical Manual, Chapter 18
[62] Compendium of Methods for the Microbiological Examination of Foods, Chapter 8 and 9.62 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[63] Compendium of Methods for the Microbiological Examination of Foods, Chapter 9.933. Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[64] Pathogenic (*Escherichia coli* O157:H7, *Escherichia coli* (Shiga toxin-producing) and non-pathogenic)
[65] Compendium of Methods for the Microbiological Examination of Foods, Chapter 9.933
[66] Association of Official Analytical Chemists (AOAC) RI #040702 (A) / AOAC-PTM 040702
[67] Association of Official Analytical Chemists (AOAC) RI 121501 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[68] Association of Official Analytical Chemists (AOAC) RI 061703 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[69] FDA Bacteriological Analytical Manual, Chapter 12 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[70] Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London:Health Protection Agency, November 2009.
[71] FDA Bacteriological Analytical Manual, Chapter 14 Accreditation: ISO/IEC 17025:2017 A2LA 3329.05
[72] FDA Bacteriological Analytical Manual, Chapter 16
[73] Set at lower limit of detection for this assay
[74] Association of Official Analytical Chemists (AOAC) RI-ELFA
[75] Association of Official Analytical Chemists (AOAC) 993.14. Accreditation: ISO/IEC 17025:2017 A2LA 2918.01
[76] Eurofins Microbiology Laboratories internal testing methodology, 2022
[77] ISO 19343:2017 (E), modified
[78] Biji KB, Ravishankar CN, Venkateswarlu R, Mohan CO, Gopal TK. Biogenic amines in seafood: a review. J Food Sci Technol. 2016 May;53(5):2210-8. doi: 10.1007/s13197-016-2224-x.

WILDTYPE

Received: 3 July 2023
Responded: 28 July 2023

Overview
This document responds to the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 3 July 2023. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests

Identity

1: Information Requested

Please provide a complete description of the current version of your production process. This would include:

- Cell lines currently used for food production and information characterizing the management and storage of those cell lines;
- The material inputs (e.g., media and any other pre-harvest inputs), if changed
- The steps of the culture process from initiation of the seed train to the harvest step, as well as the most current version of the hazard analyses and controls (e.g., analytical testing, including relative frequency) for each step based on recent production experience and any incidents (e.g., contamination) that may have occurred;
- A description of your harvest step that clarifies the point at which cell viability is no longer being maintained; and
- Structural materials (if any) introduced prior to termination of cell viability.

Where detailed information has previously been provided and remains largely unchanged, only sufficient information to clearly articulate the current version of the process is necessary. Where certain details are considered confidential, please provide a summary of this information for the disclosable safety narrative as well as the supplemental confidential appendix.

Significance: The scope of the pre-market consultation involves the steps of production from cell sourcing to harvest (i.e., the point at which the cells are no longer viable). A clear description of these steps is necessary to define the subject of the consultation. Future iterations of the process that differ substantially at one or more steps with respect to material inputs or processing operations that would alter the properties of the harvested cell material would be most appropriately addressed through a supplement to a completed consultation or a separate consultation entirely.

Cell lines: The cell line (derived from Coho salmon) described in CCC 000005 and subsequent amendments continues to be employed in Wildtype's food production process today. Following an FDA audit conducted onsite in May 2023, Wildtype has further enhanced its methods to manage and store both working and master cell banks. These procedures include consistent cell line naming conventions, color coded storage boxes / vials to minimize the opportunity for operators to thaw incorrect vials, implementation of an enterprise resource planning software to track all inputs (including cell lines) throughout the production process, and guidance for cell line characterization. For example, before submitting a vial to Wildtype's master cell bank, species confirmation via genetic barcoding or confirmation by cytochrome C oxidase I polymerase chain reaction (PCR) amplification must be

performed on DNA extracted from Wildtype cell line candidates. Additionally, specifications for pathogen testing required for cell banking are also outlined in the relevant standard operating procedure.

Material inputs: Changes to the material inputs used in Wildtype's cell culture medium are described in our response to question 3 below. There have been no other changes to pre-harvest inputs.

Culture process steps: The cell culture process from initiation of the seed train to the harvest step remains largely unchanged from that described in CCC 000005. The process is summarized sequentially in the hazard analysis in the appendix of this amendment, as well as below where we note (in bold text) the stages where there have been material changes since CCC 000005 was submitted.

1. Receiving raw ingredients: no significant change
2. Media preparation: **changes to media composition covered in response to question 3 below**
3. Cell banking: changes described above in the "cell lines" section
4. Cell thaw: no significant change
5. Seed train and proliferation: Wildtype's production process has evolved from a simple batch process to a fed-batch process. **New cell feed (bolus) inputs are included in our response to question 3 below**.
6. Cell harvest from bioreactors: no significant change
7. Scaffold production & combination with cells: **described below in "harvest step and structural materials"**
8. Formulation of finished product: **described below in "harvest step and structural materials"**

Harvest step & structural materials: The harvest process begins when cells are collected from the bioreactor via bowl centrifugation and washed with a phosphate buffered saline solution, they are frozen and stored in a -80°C freezer. While cell viability is no longer actively monitored after removal from the bioreactor, cells are not confirmed to be definitively non-viable until they are combined with the structural materials described below and subjected to a thermal process described below. For this reason, Wildtype defines the "harvested cell material" referenced by FDA in subsequent questions to comprise both cells and the structural materials used during steps 7 and 8.

Wildtype's scaffold inputs, which are introduced prior to termination of cell viability, have not changed from those disclosed in our January 17, 2023 amendment. Steps 7 and 8 are largely unchanged from the process described in CCC 000005 and subsequent amendments: namely the collection, mixing, and assembly of plant-based structural materials and cells. The most substantive change to steps 7 and 8 versus what was described in CCC 000005 is that the "maturation" stage, as it was described in CCC 000005 is currently excluded from Wildtype's process. The harvest process concludes with a validated thermal process (discussed at length in our January and May 2023 amendments) intended to render the cells non-viable and control for pathogens of concern such as *Listeria, Salmonella,* and others. Testing for adventitious agents and other contaminants discussed in this and previous amendments occurs at the conclusion of this step.

2: Information Requested:

Please provide information that characterizes the identity of the harvested cell material, including information on contaminants, proximates, nutrients, and specifications.

Significance: Due to your iteration of the production process over time that altered the stage at which harvest of the cells occurs, it would be helpful to clearly identify and characterize the harvested cell

material that is the subject of the consultation, as opposed to subsequent stages of production that incorporate non-living cell material into finished food products.

The following figures characterize the identity of representative samples of harvested cell material. Figure 1 is the result of regular adventitious agent monitoring that was intended to validate adventitious agent sampling methods - six samples of harvested cell material produced in six lots between April and June 2023 were sent to an external laboratory in June 2023 for analytical testing. Specifications are consistent with those outlined in Figure 5 of our May 3, 2023 amendment. Figure 2 provides nutritional and proximate analysis results provided by an external laboratory. All tests were conducted by accredited laboratories using methods validated for their intended purposes.

Figure 1: Contaminant analysis and specifications in harvested cell material

Parameter	Method	Specification[1]	Lot 1: 4/21/23	Lot 2: 4/28/23	Lot 3: 5/5/23	Lot 4: 5/19/23	Lot 5: 5/26/23	Lot 6: 6/9/23
Aerobic plate count	AOAC OMA 990.12	<1,000 cfu/g	<10	20	<10	20	10	<10
Yeast	AOAC OMA 2014.05	<100 cfu/g	<10	<10	<10	<10	<10	<10
Mold	AOAC OMA 2014.05	<100 cfu/g	<10	<10	<10	<10	<10	<10
Coliforms	AOAC OMA 991.14	<100 cfu/g	<10	<10	<10	<10	<10	<10
E. Coli	AOAC OMA 991.14	<20 cfu/g	<10	<10	<10	<10	<10	<10
***E. Coli* O157:H7**	AOAC RI 020801	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g
Enterobacteriaceae	AOAC OMA 2003.01	<100 cfu/g	<10	<10	<10	<10	<10	<10
Staphylococcus aureus	AOAC OMA 2003.07	<20 cfu/g	<10	<10	<10	<10	<10	<10
Bacillus cereus	FDA BAM ch. 14	<1,000 cfu/g	<100[2]	<100	<100	<100	<100	<100
Salmonella	AOAC OMA 2011.03	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g
Listeria monocytogenes	AOAC OMA 2004.02	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g
***Campylobacter* species screen**	AOAC RI 040702	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g
***Staphylococcus* enterotoxin**	AOAC 2007.06	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g
***Clostridium botulinum* toxin**	LC-MS/MS	Not detected / 50g	Not detected / 50g	Not detected / 50g	Not detected / 50g	Not detected / 50g	Not detected / 50g	Not detected / 50g
***C. Perfringens* toxin**	Eurofins internal method	<2ng / ml	<2ng / ml	<2ng / ml	<2ng / ml	<2ng / ml	<2ng / ml	<2ng / ml
Arsenic	AOAC OMA 2015.01	<50 ppb	<10	<10	<10	<10	<10	<10
Cadmium	AOAC OMA 2015.01	<20 ppb	<10	<10	<10	<10	<10	<10
Lead	AOAC OMA 2015.01	<20 ppb	<10	<10	<10	<10	<10	<10
Mercury	AOAC OMA 2015.01	<50 ppb	<10	10	10	<10	10	<10

[1] References for methods and specifications were provided in Figure 5 of the January 17, 2023 amendment.
[2] Limit of detection = 100 cfu/g

Figure 2: Proximate and nutritional analysis and specifications of harvested cell material

Parameter	Method	Specification	Lot 1: 4/14/23	Lot 2: 4/21/23	Lot 3: 5/26/23
Calories (per 100g)	Calculated (Atwater calculation)	100-200 kcal	161 kcal	161 kcal	169 kcal
Total fat	AOAC 954.02	5-20%	15.2%	14.35%	15.9%
Saturated fat	AOAC 996.06	<5%	1.7%	1.62%	1.8%
Monounsaturated fat	AOAC 996.06	<10%	8.2%	7.78%	8.7%
Polyunsaturated fat	AOAC 996.06	0-5%	3.9%	3.65%	4.1%
Trans fat	AOAC 996.06	<1%	< 0.1%	< 0.1%	< 0.1%
Triglycerides	AOAC 996.06	5-20%	14.5%	13.65%	15.2%
Protein	AOAC 990.03	3-10%	3.9%	4%	4%
Carbohydrates	Calculated	0-10%	3.5%	5.6%	3.9%
Ash	AOAC 942.05	<1%	<0.4%	<0.4%	<0.4%
Moisture	AOAC 925.09	70-85%	78%	77%	77%
Vitamin A (per 100g)	AOAC 974.29	100-500 IU	268 IU	258 IU	252 IU
Vitamin B5 (per 100g)	AOAC 945.74	0-1 mg	0.1 mg	0.1 mg	0.1 mg
Folate (per 100g)	AOAC 992.05	0-0.5 mg	0.02 mg	0.02 mg	0.02 mg
Vitamin B12 (per 100g)	AOAC 952.20	5-15 µg	8 µg	11.9 µg	8.3 µg
Vitamin D (per 100g)	LC-MS/MS (Ref: Huang et al. Rapid Commun, *Mass Spectrum* 2014, 28	0-10 IU	<4 IU	<4 IU	<4 IU

Food Safety Assessment

3: Information Requested:

As appropriate, please provide an updated model for anticipated exposure to medium components based on the most current version of your production process.

Significance: Mass-balance calculations incorporated into exposure estimates may change significantly depending on the design of the production process.

Since submission of CCC 000005, optimization of Wildtype's cell culture media formulation has resulted in the discontinuation of insulin and fetal bovine serum. Several inputs not listed in the original dossier are now part of the media formulation, and are summarized below. Changes to Wildtype's process did not result in other material changes to the previously reported medium component exposure analysis. Substances that were either permitted by federal regulation to be used in food without limitation or permitted to be directly added to food in a manner consistent with Wildtype's use were not subjected to further safety assessments beyond a mass balance calculation, which was performed to estimate the concentration in the finished product as well as estimated daily intake (EDI) using the method described in CCC 000005. In general, the levels of all of the new medium inputs were calculated to be trivially small and therefore practically not detectable in the finished product. The absence of meaningful exposure to these substances in the finished food product provides assurance that their use is safe. For other inputs, Figure 3b summarizes available scientific literature or reference to exposure via conventional food, when available, substantiating our general claim that the levels of cell culture medium inputs in the finished product are many orders of magnitude below established safety thresholds found in the scientific literature (e.g., for all practical purposes, not present).

Page 5 of 20 removed.

Removed page contains trade secret and confidential commercial information exempt from disclosure under exemption 4 of the Freedom of Information Act.

As cell culture media components, each of these is subjected to dilution step 8, referenced in CCC 000005. The calculations follow those set forth in response to question 24 of the January 17, 2023 amendment (p. 32-33).

Ascorbic acid is used as an example here:

Dilution steps: 8, referenced in CCC 000005
Initial concentration: 200 mg/L
Calculated final concentration: 200 mg/L x 1E-12 {step 8} = 2E-10 mg/L

The final concentration (2E-10 mg/L) is multiplied by the 90th percentile average intake of salmon (1.54 g /kg bw /day) to obtain the 90th percentile estimated intake of the compound (2E-10 mg/L) x (1.54 g/kg bw /day) x (1L g / 1000 g) = 3.08E-13 mg/kg bw/day

To obtain the daily intake in mg/day, the final concentration (2E-10 mg/L) is multiplied by the 90th percentile average intake of salmon (109 g/day) to obtain the 90th percentile estimated intake of the compound (2E-10 mg/L) x (109 g/day) x (1L g / 1000g) = 2.18E-11 mg/day.

Controls

4: Information Requested:

Please provide an affirmative statement that you will test for the presence of toxic heavy metal contaminants in the harvested cell material consistent with your specifications on a more frequent ongoing basis, rather than "at least twice in the next 12 months" as clarified in the May 3, 2023, amendment. We recognize that this may include adjustments in frequency over time based on production experience with various sources of medium components.

Significance: The presence of toxic heavy metals in foods is undesirable and should be limited to levels as low as possible. In light of the limited experience with the potential for bioaccumulation across varied scales and culture feedstock sources, regular, ongoing monitoring is appropriate at this time.

Wildtype will test for the presence of toxic heavy metal contaminants in the harvested cell material consistent with the company's specifications on a more frequent regular basis, rather than at least twice per year. Testing for heavy metal contaminants will be conducted when there are significant changes to inputs (e.g. cell culture medium components), processes, or production machinery. Additionally, ongoing monitoring more than twice per year will help mitigate the potential for bioaccumulation of these potential contaminants. For example, six lots produced between April and June, 2023 were tested as part of this regular monitoring in June 2023 and reported above in Figure 1. Heavy metals levels in all six lots were within specification.

5: Information Requested:

Please provide an affirmative statement that you will test for the presence of all of the microorganisms identified as part of the "ongoing" analysis of the harvested cell material in Figure 5 of the January 17, 2023, amendment on a more frequent basis, rather than "at least twice per year" as clarified in the May 3, 2023, amendment, consistent with your specifications, which may include adjustments in frequency over time based on production experience in different configurations.

Significance: These microorganisms may be introduced from the environment or personnel at various stages of production. In light of the limited experience with implementation of your culture process at various scales and levels of automation, regular, ongoing monitoring is appropriate at this time.

Wildtype will test for the presence of all microorganisms identified as part of the "ongoing" analyses in Figure 5 of the January 17, 2023 amendment in the harvested cell material on a more frequent regular basis, rather than at least twice per year as clarified in our May 3, 2023 amendment, consistent with our specification, which may include adjustments in frequency over time based on production experience in different configurations.

For example, although Figure 5 in our amendment from May 3, 2023 indicates "ongoing" testing would be conducted at least twice a year, every finished good production lot produced in 2023 has been subjected to the tests outlined in the "ongoing" column in Figure 5. Results from six such lots are reported in Figure 1 above. Ongoing monitoring for spore formers such as *Bacillus cereus, Clostridium perfringens, and Clostridium botulinum* has also been conducted on 2023 production lots.

6: Information Requested:

In the May 3, 2023, amendment, you state "*Bacillus cereus* testing is part of standard microbiological testing of every lot during the 90-day "baseline" period; testing for cereulide is obviated by consistent testing that reveals the absence of *Bacillus cereus* contamination, as the bacterium is required for toxin production." For addition to the disclosable safety narrative, please discuss how you intend to monitor or otherwise control for the presence of spores from *B. cereus*.

Please provide an affirmative statement that you will monitor or otherwise control for the presence of spores and toxins from microorganisms (e.g., *B. cereus*, *Staphylococcus aureus*) in the harvested cell material on a more frequent ongoing basis, rather than during a 90-day baseline period or "one time," respectively, as clarified in the May 3, 2023, amendment, which may include adjustments in frequency over time based on production experience in different configurations.

Significance: Spores and heat stable toxins (i.e., staphylococcal enterotoxin) are not amenable to direct thermal control steps. Further, as noted previously, reports in the literature that note that growth of S. aureus and production of staphylococcal enterotoxins may be decoupled (i.e., active growth of S. aureus may not be necessary for enterotoxin production), while foodborne illness attributed to S. aureus is often associated with growth in protein-rich food, including fish and fish products.[10,11]

Wildtype will monitor or otherwise control for the presence of spores and toxins from microorganisms (e.g., *B. cereus*, *Staphylococcus aureus*) in the harvested cell material on a more frequent ongoing basis, rather than during a 90-day baseline period or "one time," respectively, as clarified in our May 3, 2023, amendment, which may include adjustments in frequency over time based on production experience in different configurations. For example, six such production lots were tested for both *B. cereus* and *Staphylococcus aureus* in the six representative production lots listed in Figure 1.

In terms of how we will monitor or otherwise control for the presence of spores from *B. cereus,* as noted in Figure 5 of the January 17, 2023 amendment, the *B. cereus* test is an agar plate count, providing favorable conditions for potentially contaminating spores to sporulate and propagate, thus resulting in

[10] Schelin, J. et al. (2011). The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. Virulence, 2(6), p. 580-592. doi: 10.4161/viru.2.6.18122
[11] Grispoldi, L. et al. (2021). Staphylococcus aureus enterotoxin in food of animal origin and staphylococcal food poisoning risk assessment from farm to table. Italian Journal of Animal Science, 20(1), p. 677-690. doi: 10.1080/1828051X.2020.1871428

detection of any *B. cereus spores*. To date, there has not been any testing result out of specification for spores and toxins from microorganisms in any production lot.

7: Information Requested:

Information establishing the efficacy of a control step (such as a thermal process step) should ideally be established by a scientific validation study that is designed to ensure an appropriate reduction in the number of pathogenic bacteria of public health concern (e.g., six-log reduction of *Listeria monocytogenes*), and that the target thermal lethality is achieved in every unit of product (e.g., heat penetration and temperature distribution study). Generally, *L. monocytogenes* is recommended as the target pathogen for seafood because it is regarded as the most heat-tolerant foodborne bacterial pathogen of concern that does not form spores.[12,13] Where you are relying on study data to demonstrate the efficacy of a secondary post-harvest control step to address hazards associated with a culture process step, please identify any potentially relevant limitations in your study design and steps you have taken to address these limitations, including additional control steps.

Significance: In some cases, hazards identified during a culture process step may be reasonably addressed by post-harvest controls. However, to the extent that there are limitations in data demonstrating the efficacy of such a control and limited experience with implementation of the control, secondary or supplementary controls (such as ongoing testing/monitoring) may be appropriate.

The validation study described in our amendment submitted to FDA on May 3, 2023 (Table 1) demonstrated at least a six-log reduction of *Listeria monocytogenes* in Wildtype's finished food product. For example, at 70°C, a six log reduction of *L. monocytogenes* was achieved in less than 12 minutes, well under the time currently employed in Wildtype's lethal step. Additionally, internal heat step validation studies confirm that target thermal lethality is achieved in every unit of product by following the steps outlined in Wildtype's standard operating procedures and master batch records for the lethal processing step.

The researchers at Texas Tech University who carried out the validation study identified one significant limitation to the study, namely the non-extensibility of this control to spore formers. Considering this limitation, other controls and measures are employed to limit spore formers including plant sanitation, personal protective equipment, GMPs, and testing of the finished product as described in our response to questions 2 and 6 above.

Given the novelty of Wildtype's food production technology, ongoing testing and monitoring is employed as a verification method to ensure that thermal process and other production controls are effective in mitigating adventitious agents.

Points of Clarification

8: Information Requested:

On page 1 of the May 4, 2023, amendment, you indicate that the vendor for the source animals does not provide a health certificate, but does conduct a number of tests to characterize potential health issues.

[12] More information on FDA's recommendations for cooking by seafood processors can be found in Chapter 16 of FDA's Fish and Fishery Products Hazards and Controls guidance.
[13] The submission from Wildtype incorrectly refers to the guidance when it states that FDA guidelines recommend cooking finfish to a minimum internal temperature of 63°C (145°F). The correct reference for this recommendation is the FDA Food Code Section 3-401 which is for retail operations and not for seafood processors. Additionally, in Annex 3 of the Food Code the target pathogen is Salmonella serovars since the cook temperature is for more than only seafood and is based on USDA guidance.

If available, please provide information on specific tests that the supplier conducts to detect parasites, spores, and pathogenic bacteria.

After submitting our May 3, 2023 amendment, we received the following health certificate from the Washington Department of Fish and Wildlife (contact information has been redacted for privacy). Testing information is indicated on the certificate.

Figure 4: Health certificate from Washington Department of Fish & Wildlife

State of Washington
DEPARTMENT OF FISH AND WILDLIFE
Mailing Address: 600 Capitol Way N, Olympia WA 98501-1091, (360) 902-2200, TDD (360) 902-2207
Main Office Location: Natural Resources Building, 1111 Washington Street SE, Olympia WA

Fish Health Report: Out-of-Basin Transfer Request

Date of Report:	5/5/23	Shipment Date:	9/28/2018
Shipped from:	WA Dept Fish and Wildlife Issaquah Hatchery 125 W Sunset Way Issaquah, WA, 98027	Recipients:	Wild Type, Inc. Justin Kolbeck [redacted]
WDFW Contacts:	Travis Burnett (FH SP 3) travis.burnett@dfw.wa.gov Darin Combs (FH SP 4) darin.combs@dfw.wa.gov (425)-391-9094	Wild Type Contact: Justin Kolbeck	[redacted]
Fish Stock:	**CO:NA:ISSA:17:M** **Coho Salmon** **10 Juvenile**	Source:	Issaquah Creek

Eggs were collected from mixed broodstock from the run of the river trap at Issaquah Hatchery, where they were fertilized, disinfected and raised until eyed. After rearing for roughly nine months, juvenile coho were shipped to Wild Type, Inc. At spawning, WDFW Fish Health collected ovarian fluids and kidney/spleen tissue pools for virology testing. WDFW performed routine fish health monitoring and available reports are provided to Wild Type. Fish deemed healthy at time of shipment.

Past 3 year virology history for this broodstock: OIE Reportable Pathogen Testing

Note: Samples processed by the WDFW Fish Health Laboratory in accordance with AFS Blue Book standards. EPC and CHSE cell lines used.

Facility	Stock	Species	Date of Collection	Results	Life Stage	Number of Samples				Inoculation Date	BP
						OF	Pool	K/S	Pool		
Issaquah Hatchery	Issaquah	COHO	10/31/17	**NEV**	Adult	60	12	60	12	11/02/17	N
Issaquah Hatchery	Issaquah	COHO	11/08/16	**NEV**	Adult	60	12	60	12	11/10/16	N
Issaquah Hatchery	Issaquah	COHO	11/03/15	**NEV**	Adult	60	12	60	12	11/04/15	N

Table Key: OF -Ovarian Fluid; K/S - Kidney/Spleen; NEV - No evidence of virus; BP - Blind passage performed

Signature of approving veterinarian: [redacted] Date: 05.05.2023

Megan Finley, DVM (VT)
WDFW Aquatic Veterinarian, Region 2/Wenatchee
megan.finley@dfw.wa.gov Cell: (509) 607-6243

9: Information Requested:

On page 2 of the May 4, 2023, amendment, you note that "… germination of remaining *Clostridium botulinum* spores would result in a cytotoxic contamination of the cultures that would be detected via routine pH monitoring and microscopy." Please provide a brief statement explaining how contamination with this microorganism would be expected to affect pH.

Although the oxygen-rich environment of cell production renders *Clostridium botulinum* contamination practically implausible, the predicted effect of this organism's growth on pH is based upon observations of pH in a reactor used to grow *Clostridium botulinum* in a glucose-containing culture medium. For example, Figure 1 of Siegel and Metzger[14] demonstrates dramatic pH changes in the first 12 hours of *C. botulinum* culture, which would be readily detected by the pH monitoring systems implemented in Wildtype's production vessels.

10: Information Requested:

On Page 5 of the May 4, 2023, amendment, you report as part of your sample environmental monitoring data, "Other processing area swabs within specification: 98%." Please provide a brief statement explaining whether and when any mitigation measures and actions would be implemented based on a test result that does not meet the set specification.

Wildtype employs the recommendations outlined in FDA's Control of *Listeria monocytogenes* in Ready-To-Eat Foods.[15] In the event a test result does not meet the set specification, Wildtype's food safety team conducts an investigation, including a root cause analysis, to determine the appropriate corrective action. Example steps (adapted from FDA's guidance referenced above) include, but are not limited to:

- Examining the site that yielded the out of specification finding, as well as the surrounding areas.
- Evaluating whether adequate sanitation preventive controls are established along with the cleaning tools, techniques, and procedures followed.
- Reviewing Wildtype's food safety plan and its implementation to determine if there are any design or execution flaws, and modifying the plan as required.
- Conducting intensified environmental sampling and testing of sites that represent potential sources of contamination.
- Testing areas upstream of the contamination source to identify source.
- Checking maintenance records for modifications or repairs to equipment.
- Interviewing and observing sanitation, maintenance, and production personnel to determine whether appropriate procedures are being followed and whether modifications are required to prevent contamination
- Reviewing traffic patterns, equipment layout, and adherence to personnel hygiene procedures.
- Taking appropriate corrective actions based on the findings of the above activities, such as intensified cleaning/sanitization followed by re-testing, vector swabbing, holding product until the investigation is complete, and careful assessment before product release.

[14] Siegel LS, Metzger JF. Effect of fermentation conditions on toxin production by Clostridium botulinum type B. *Appl Environ Microbiol.* 1980 Dec;40(6):1023-6. doi: 10.1128/aem.40.6.1023-1026.1980. PMID: 7006503; PMCID: PMC291715. Link to the original manuscript may be found here.
[15] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry - Draft Guidance, January 2017.

WILDTYPE

Appendix: Updated hazard analysis and controls

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
1	Receiving Raw Materials	Biological	Potential pathogens (e.g., *Salmonella, L. monocytogenes)* present in media & scaffold inputs	x		Scaffold/media-borne pathogens may survive into final product	**Process Preventive Controls** Thermal process in subsequent step inactivates potential pathogens Ongoing adventitious agent testing (initially every lot per "ongoing" column in Wildtype's May 3, 2023 amendment) as well as ongoing monitoring of heavy metals and spore formers. For brevity, this verification method is abbreviated as "ongoing adventitious testing" in subsequent steps in this hazard analysis.		x
		Biological	Expired materials may be provided by raw material suppliers	x		Expired materials may introduce pathogens into products	**Supply Chain Preventive Control** COAs and expiration dates inspected for each lot	x	
		Chemical	Potential for undeclared allergens in scaffold and media inputs; incorrect materials sent by the vendor	x		Suppliers may inadvertently include undeclared allergens by cross-contact, incorrect labeling.	**Supply Chain Preventive Control** - Scaffold suppliers pass through supplier qualification program prior to using input - COAs inspected for each lot - Record of allergen statement from the vendor, physical inspection of material along with their label	x	
		Physical	Potential packing/shipping materials in scaffold inputs	x		Damaged packaging or shipping materials	**Process Preventive Controls** - Visual inspection of all packages - If damage to primary package; lot is rejected	x	

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
2	Media Preparation	Biological	Potential for media sterilization failure allowing growth of pathogens (e.g., *Salmonella, L. monocytogenes)*		x	Media prep MBRS (e.g., 044 and 048) include strict sterilization requirements In the event that sterilization failed, pathogens would outcompete or affect cell growth (detectable via real-time monitoring), leading to the destruction of the batch. Ongoing adventitious agent testing in subsequent step			
		Chemical	Potential for inclusion of incorrect media components		x	SOP-012 requires incoming material inspection for all media components, which includes certificate and allergen confirmation Media prep MBRs (e.g., 044 and 048) require confirmation of lot # and expiration for each input			
		Physical	Introduction of foreign material such as metal or glass fragments during media mixing step		x	Sterile filtration process is included in the media preparation batch records with a 0.2 µm filter. Each final product is passed through the X-ray in step 9			

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
3	Cell banking	Biological	Potential introduction of microorganisms in cell banks (*Salmonella, L. monocytogenes, Staphylococcus aureus*) from the environment/personnel handling		x	Ongoing adventitious agent testing: Per SOP-002, cell vials tested via 3rd party laboratory required to be free from microbial contamination before releasing to cell banks			
		Chemical	Potential for unapproved food additives (freezing agents) in product		x	Mass balance calculations and analytical testing of finished product shows absence of freezing agents.			
		Chemical	Potential for cross contamination between different species during storage, undesirable species identified as part of the vial		x	Per SOP-002, MCBs and WCBs are clearly labeled and color coded. Cell vials with different allergens are not stored in same liquid nitrogen storage			
		Physical	None						

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
4	Cell Thaw	Biological	Potential introduction of microorganisms in cell culture (*Salmonella, L. monocytogenes, Staphylococcus aureus*) from the environment/personnel		x	Pathogens would outcompete cell growth - pathogens controlled by monitoring for contamination in each batch Subsequent lethal step Ongoing adventitious agent testing at subsequent step			
		Chemical	Potential introduction of non-labeled allergens		x	MBR-031 requires operators to affix labels from thawed vials to MBR and a secondary verifier Cell vials with different allergens are not stored in same liquid nitrogen storage			
		Chemical	Potential for thawing incorrect cell line for production		x	SOP-002 (cell banking) includes step-by-step instructions and controls to prevent thawing incorrect vial			
		Chemical	Potential for unapproved food additives (freezing agents) in product		x	Mass balance calculations and analytical testing of finished product shows absence of freezing agents.			
		Physical	None						

5	Seed train and cell proliferation	Biological	Potential growth of pathogens from the environment / human contact. Utensils/tools, bioreactor, or any equipment contaminated and not cleaned.	x		Cell culture conditions in flasks and bioreactors are amenable to pathogen growth	**Sanitation Preventive Control** Environmental monitoring program, master sanitation schedule, & GMPs mitigate environmental pathogens **Process Preventive Controls** Upstream production MBRs include aseptic techniques, process parameters to record and monitor DO changes as an indicator for contamination. Dissolved oxygen drops of >30% over an 8-hour period in bioreactors are determined to be at risk for contamination and subjected to further screening including microscopy. For shake flasks, turbidity is visually inspected at least 5x / week as a sign for contamination. If contaminated then cultures are terminated. Ongoing adventitious agent testing at subsequent step	x	
		Chemical	Cleaning chemical residue may be present in bioreactor	x		Clean-in-place chemistry may not be adequately rinsed and removed following CIP process	**Process Preventive Control** Cleaning development study is performed which determines the cleaning process. Cleaning chemistry removal and cleaning effectiveness is verified by collection of final rinse samples which are tested for pH, conductivity, ATP and visual inspection. Cleaning verification testing is performed as part of each bioreactor cleaning. Passing results are required for releasing the equipment for the next production run.	x	
		Physical	Potential for metal or glass fragments	x		Metal-to-metal contact inside a bioreactor or broken glass probes may produce metal or glass fragments	**Process Preventive Control** X-ray (conducted in a subsequent step)		x

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
6	Cell Harvest from bioreactors	Biological	Potential growth of pathogens such as *Salmonella, L. monocytogenes, Staphylococcus aureus*	x		Pathogens, if present in the environment, have the opportunity to be introduced however, the process is short and GMPs are followed throughout the process	**Process Preventive Controls** Thermal process in subsequent step inactivates potential pathogens Ongoing adventitious agent testing at subsequent step		x
		Chemical	None						
		Physical	None						

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
7	Scaffold production and combination with cells	Biological	Potential introduction of pathogens such as *Salmonella, L. monocytogenes, Staphylococcus aureus* from scaffold raw ingredients and unclean utensils/tools		x	Scaffold production occurs in high-care area subject to EMP program, enhanced gowning, and hygienic conditions controlled through master sanitation schedule; Subsequent thermal kill step. Ongoing adventitious agent testing at subsequent step			
		Chemical	Potential introduction of unapproved chemicals in scaffold inputs		x	Supply chain approval process (PGM 002) for ingredient suppliers including a letter of guarantee. Raw materials are taken out of their original packaging and transferred to another container are labeled with correct item/ lot / allergen / expiry. Operators are trained to only use materials marked with a green sticker labeled "approved" by Quality for inclusion in the finished product.			
		Chemical	Potential unlabeled seafood or plant allergens present in the product	x		Salmon is present in the finished product. Allergenic plant-based inputs are used as an input in the scaffold	**Allergen Preventive Control** Ensure all allergens are properly labeled (conducted in a subsequent step)		x
		Physical	Potential introduction of foreign material - metal fragments, glass pieces, plastic, wood, piece of glove, hair, jewelry, etc. during manual scaffold creation	x		Scaffold formulation is currently assembled by hand creating the potential for the introduction of physical hazards	**Sanitation Preventive Control** - Gowning & hygiene requirements limit operator physical hazards - Sanitation program limits environmental physical hazards - X-ray (subsequent step)	x	

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
8	Formulation of finished product (Thermal processing - CCP applied at this step)	Biological	Survival of pathogens such as *Salmonella, L. monocytogenes, Staphylococcus aureus* inside the ready-to-eat product	x		Pathogens conveyed from salmon cells or scaffold inputs could grow in the finished product	**Process Preventive Controls** Finished product is heated to lethal temperature per validated process control within vacuum-sealed bag Ongoing adventitious agent testing conducted during this stage	x	
		Biological	Potential spore forming organisms such as *Bacillus cereus* during cooling step		x	Time in danger zone is minimized and ongoing monitoring for spore formers			
		Chemical	Potential for plastic leaching from primary packaging		x	Packaging tested for leaching -- tests were negative.			
		Physical	Potential introduction of foreign material - metal fragments, glass pieces, plastic, wood, piece of glove, hair, jewelry, etc. during manual scaffold creation	x		Scaffold formulation is currently assembled by hand creating the potential for the introduction of physical hazards	**Sanitation Preventive Control** - Gowning & hygiene requirements limit operator physical hazards - Sanitation program limits environmental physical hazards **Process Preventive Control** X-ray (subsequent step)	x	

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
9	Finished product packaging and labeling (X-ray inspection - CCP applied at this step)	Biological	None						
		Chemical	Potential for allergens not properly labeled on primary packaging	x		Label printer may not include required allergen disclosures	**Allergen Preventive Control** Verification that allergens are present on pre-printed label (in MBR)	x	
		Physical	Risk of introduction of any foreign material during packaging or previous production steps	x		Visual inspection during previous process steps may miss small fragments present in the finished product	**Process Preventive Control** Inspection of each product under the in-line x-ray machine	x	

WILDTYPE

Sr. no.	Ingredient / Processing Step	Identify potential food safety hazards introduced, controlled or enhanced at this step		Do any potential food safety hazards require a preventive control?		Justify your decision for previous column	What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard? *e.g. Process including CCPs, Allergen, Sanitation, Supply-Chain, other preventive control*	Is the preventive control applied at this step?	
		Hazard Type	Hazard Name	Yes	No			Yes	No
10	Storage and distribution	Biological	Improper / damaged packaging leaves finished product exposed to environmental pathogens	x		Products that were improperly packaged or damaged may allow the infiltration and subsequent growth of environmental pathogens; thus requiring a process control.	**Process Preventive Controls** Finished product & packaging inspected by operator during production as per MBR Operator/QA to verify primary packaging is undamaged via seal integrity test prior to insertion in secondary/tertiary packaging Temperature mapping/monitoring of freezer/refrigerator for storage Planned maintenance activities to be performed regularly on the freezer/refrigerator	x	
		Chemical	None						
		Physical	Introduction of foreign material due to poor packaging /handling, damage to product during storage and distribution, storage areas unclean	x		Poor packaging and handling with unclean storage risks contaminating finished products with environmental pathogens	**Process Preventive Controls** PGM-006 Sanitation Program for adequate cleaning in storage areas Stored product areas / warehouse inspection procedures Shipping inspection to verify finished product	x	

Received: 1 November 2023
Responded: 24 January 2024

Overview
This document responds to the request for additional information re. CCC 000005 transmitted by FDA to Wildtype on 1 November 2023. For ease of reference, FDA's original questions are reproduced in black text and Wildtype's responses appear below in blue text.

Substantive Information Requests

Identity

1. Information Requested

On page 2 of the in the July 28, 2023, amendment, in response to question 1, you state that cells are removed from the bioreactor and "frozen and stored in a -80°C freezer." You go on to state, "While cell viability is no longer actively monitored after removal from the bioreactor, cells are not confirmed to be definitively non-viable until they are combined with the structural materials described below and subjected to a thermal process described below. For this reason, Wildtype defines the "harvested cell material" referenced by FDA in subsequent questions to comprise both cells and the structural materials used during steps 7 and 8." Later you state "the "maturation" stage, as it was described in CCC 00005 is currently excluded from Wildtype's process." We note the presence of the maturation stage was fundamental to the subject of previous discussions, including the meeting of May 26, 2023, and related follow-up, between FDA and Wildtype on the boundaries of the harvest process, and how that pertains to the scope of cell culture consultation.

In the absence of data demonstrating cell viability post -80°C freeze and subsequent thaw and based on FDA's understanding of the underlying nature of post-thaw cellular viability in the absence of cryoprotectants, FDA sees no evidence to conclude the cells could be viable past the freeze stage after removal from the bioreactor. While FDA is willing to entertain arguments that there may be times when the harvest process includes steps beyond removal from the bioreactor, the process, as currently described, contains no steps where the cells are growing, proliferating, or maturing. In the absence of information that the cells are viable, and that viability is necessary to future steps, FDA has concluded that the scope of the cell culture consultation ends at the point of cell removal from the bioreactor. FDA will not consider data and information presented for steps after this point, including analytical data from thawed cells combined with structural materials, except as they pertain to safety questions that apply to steps within the scope of the cell culture consultation.

We accept this scope of the cell culture consultation.

Product Characterization

2: Information Requested

FDA has determined that, based on your updated production process, harvest is the point where the cells are removed from the bioreactor. Please clarify, if any of the test data provided, which notes the tests were performed on the harvested material, were performed on the cells at the point of harvest from the bioreactor. If not, please provide new data to demonstrate the levels of proximates,

micronutrients, fatty acids, heavy metals, and adventitious agents of concern in the cells removed from the bioreactor (i.e., the point of harvest), prior to freezing and storage at -80°C.

Requested data follow for cells at the point of harvest from the bioreactor. COAs are in Appendix 1 (starting on page 11).

Figure 1: Proximates, fatty acids, and micronutrients for cells at the point of harvest from bioreactor

Parameter	Method[1]	Specification	Lot 1: 11/9/23	Lot 2: 11/16/23	Lot 3: 11/30/23
Calories (per 100g)	CFR - Atwater calculation	40 - 100 kcal	58 kcal	49 kcal	53 kcal
Total fat	AOAC 954.02	0.5 - 12%	1.82%	1.71%	1.54%
Protein	AOAC 990.03; AOAC 992.15	5 - 25%	11.06%	10.19%	10.69%
Carbohydrates	CFR 21 - Calculated	<5%	0.72%	<0.5%	<0.5%
Ash	AOAC 942.05	<5%	1.62%	1.93%	2.05%
Moisture	AOAC 925.09	75 - 90%	85.4%	87.1%	86.0%
Saturated fat	AOAC 996.06	<2%	0.29%	0.24%	0.27%
Monounsaturated fat	AOAC 996.06	<5%	0.55%	0.51%	0.52%
Polyunsaturated fat	AOAC 996.06	<5%	0.25%	0.12%	0.13%
Trans fat	AOAC 996.06	<1%	0.06%	0.04%	0.04%
Triglycerides	AOAC 996.06	<5%	1.2%	0.95%	1.00%
Total omega 3 isomers	AOAC 996.06	<2%	0.12%	<0.05%	<0.05%
Total fatty acids	AOAC 996.06	0.5 - 12%	1.15%	0.91%	0.96%
Vitamin A (per 100g)	AOAC 974.29	<100 IU	<30 IU	<30 IU	<30 IU
Vitamin B5 (per 100g)	AOAC 945.74	<5 mg	1.35 mg	1.8 mg	1.28 mg
Folate (per 100g)	AOAC 992.05	<1 mg	0.078 mg	0.0915 mg	0.0441 mg
Vitamin B12 (per 100g)	AOAC 952.20	<1 mg	248 µg	201 µg	230 µg
Vitamin D2 & D3 (per 100g)	Huang et al. Rapid Commun, *Mass Spectrum* 2014, 28	<12,000 IU[2]	6,760 IU	9,790 IU	9,210 IU

[1] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[2] Testing of finished products made with these cells demonstrates Vitamin D levels <4 IU, consistent with previous data provided to FDA during this consultation process (see Appendix 5, page 79)

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Figure 2: Heavy metals & adventitious agents of concern for cells at the point of harvest from bioreactor versus conventional Coho salmon

Parameter	Method[3]	Specification[4]	WT Lot 1: 11/9/23	WT Lot 2: 11/16/23	WT Lot 3 11/30/23	Conv. Coho 1[5] (2312868-2)	Conv. Coho 2 (2312868-3)	Conv. Coho 3[6] (2312936-2)
Aerobic plate count	AOAC 966.23	<100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	120,000 cfu/g	700 cfu/g	<10 cfu/g
Yeast	FDA BAM Ch. 18	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	100 cfu/g	20 cfu/g	<10 cfu/g
Mold	FDA BAM Ch. 18	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	30 cfu/g	10 cfu/g	10 cfu/g
Coliforms	CMMEF Chapter 9.933	<100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	20 cfu/g	10 cfu/g	<10 cfu/g
E. coli	CMMEF Chapter 9.933	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
***E. coli* O157:H7**	AOAC-RI 031002	Not Detected/25g	Not Detected/25g	Not Detected/25g	Not Detected/25g	Not tested	Not tested	Not tested
Enterobacteriaceae	CMMEF Ch. 9.62	<20 cfu/g	10 cfu/g	<10 cfu/g	<10 cfu/g	60 cfu/g	20 cfu/g	<10 cfu/g
Staphylococcus aureus	FDA BAM Ch. 12	<20 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
***Bacillus cereus* organism**	FDA BAM Ch. 14	<100 cfu/g[7]	<10 cfu/g	<10 cfu/g	<10 cfu/g	<100 cfu/g	<100 cfu/g	<100 cfu/g
Salmonella spp	AOAC-RI 121501	Not detected / 25g[8]	Not detected / 25g	Not detected / 25g	Not detected / 25g	Negative / 25g	Negative / 25g	Negative / 25g
Listeria monocytogenes	AOAC-RI 061703	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Negative / 25g	Negative / 25g	Negative / 25g
***Campylobacter* spp screen**	AOAC-PTM 040702	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Negative / 25g	Negative / 25g	Negative / 25g
***Staphylococcus* enterotoxin**	AOAC 2007.06	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not detected / 25g	Not tested	Not tested	Not tested
***C. botulinum* organism**	FDA-BAM, 8th ed.	Negative / 8g	Negative / 8g	Negative / 8g	Negative / 8g	Not tested	Not tested	Not tested
***C. perfringens* organism**	ISO 7937	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Arsenic	AOAC 2011.19, 993.14 and 2015.01	<100 ppb	56.5 ppb[9]	81.0 ppb	97.5 ppb	70 ppb	270 ppb	90 ppb
Cadmium	AOAC 2011.19, 993.14 and 2015.011	<20 ppb	5.8 ppb	<5 ppb	<5 ppb	<10 ppb[10]	<10 ppb	<10 ppb
Lead	AOAC 2011.19, 993.14 and 2015.01	<20 ppb	<5 ppb	<5 ppb	<5 ppb	<10 ppb[11]	<10 ppb	<10 ppb
Mercury	AOAC 2011.19, 993.14 and 2015.01	<20 ppb	<5 ppb	<5 ppb	<5 ppb	10 ppb	50 ppb	<10 ppb

[3] All methods are validated for their intended purposes and are carried out by an accredited external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[4] References for methods and specifications were provided in Figure 5 of the January 17, 2023 amendment.
[5] Three separate samples of conventional Coho salmon are presented here as a comparison to cultivated salmon cells. Corresponding COAs are located in Appendix 1 (starting on page 37). The names of the suppliers have been redacted for privacy. The numbering scheme in the title of Figure 2 (e.g., 2312868-2) corresponds to the testing laboratory's (Aemtek) sample number for ease of reference.
[6] Advertised on the vendor's website as "sashimi quality"
[7] Limit of detection is 10 for Eurofins (e.g., salmon cells harvested from the bioreactor, such as WT Lot 1, 2, and 3 above); 100 for tests carried out by Aemtek (e.g. conventional Coho)
[8] Aemtek reports Salmonella, Listeria monocytogenes, and Campylobacter as "negative" / 25g rather than "not detected" / 25g as does our other external testing laboratory, Eurofins
[9] Testing of finished products made with these cells shows that heavy metals are below the limit of detection (see Appendix 4, page 79).
[10] Limit of detection for the testing lab used for conventional Coho is 10 ppb for cadmium (applies to samples 2312868-2, 2321868-3, and 2312936-2)
[11] Limit of detection for this testing lab used for conventional Coho is 10 ppb (applies to samples 2312868-2, 2321868-3, and 2312936-2)

Controls

3: Information Requested

As discussed in our email dated September 27, 2023, please provide copies of the certificates of analyses (COAs) or any analytical reports for the analytical testing results reported in Figures 1 and 2 of the July 28, 2023, amendment (pages 3 and 4).

COAs are included in Appendix 2 (starting on page 43) of this document.

4: Information Requested

As discussed in our email dated September 27, 2023, please provide the results from updated fatty acid analytical testing performed since the July 28, 2023, amendment. Please also provide copies of the COAs or any analytical reports for the updated analytical testing performed.

Fatty acid testing for the harvested cell material is included in Figure 1 with corresponding COAs in Appendix 1 (starting on page 11). Results from fatty acid testing for three non-consecutive finished product batches (harvested cell material combined with scaffold) follow. Corresponding COAs with full results are in Appendix 3 (starting on page 70) of this document.

Figure 3: Fatty acid analytical testing for finished product

Parameter	Method[12]	Specification	Lot 1: 8/4/23	Lot 2: 10/4/23	Lot 3: 10/6/23
Total fatty acids	AOAC 954.02	5-20%	13.02%	13.28%	13.96%
Total saturated fatty acids	AOAC 996.06	<5%	1.65%	1.67%	1.73%
Total monounsaturated fat	AOAC 996.06	<10%	7.77%	7.87%	8.29%
Total polyunsaturated fat	AOAC 996.06	0-5%	3.58%	3.73%	3.92%
Total trans fatty acids	AOAC 996.06	<1%	0.02%	<0.02%	0.02%
Total fats as triglycerides	AOAC 996.06	5-20%	13.60%	13.87%	14.58%
Total omega 3 isomers	AOAC 996.06	<10%	1.87%	2.05%	2.16%
Total omega 6 isomers	AOAC 996.06	<10%	1.68%	1.66%	1.74%
Total omega 9 isomers	AOAC 996.06	<10%	7.49%	7.62%	8.02%

All methods are validated for their intended purposes and are conducted by an accredited external laboratory.

5: Information Requested

On page 3 of the July 28, 2023, amendment, you report the specification for *Bacillus cereus* as <1000 colony forming units (CFU)/g. The results from the analytical testing of the 6 batches reported in Figure 1 for *B. cereus* are <100 CFU/g, which you note is the limit of detection for the selected analytical method. Considering that the *B. cereus* emetic toxin cereulide, as well as *B. cereus* spores, are not amenable to direct thermal control steps[13,14]; that reports in the literature note the infective dose of *B. cereus* may start at 103 CFU/g of food; that this product is described as "ready-to-eat" in the January 17, 2023, amendment; and that you have reported levels much lower than your proposed specification, we request that you lower your specification for *B. cereus* to as low as can be reasonably obtained.

Further, you report the specification for *Enterobacteriaceae* as <100 CFU/g, while the results from the analytical testing reported in Figure 1 are <10 CFU/g. As *Enterobacteriaceae* is a large family of

[12] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[13] Rajkovic, A., et al. (2008). Heat resistance of Bacillus cereus emetic toxin, cereulide. Letters in Applied Microbiology, 46(5), p. 536-541. doi: 10.1111/j.1472-765X.2008.02350
[14] Yang, S., et al. (2023). Cereulide and emetic Bacillus cereus: Characterizations, impacts and public precautions. Foods, 12(4). doi: 10.3390/foods12040833

microorganisms that includes notable foodborne pathogens, and the fact that you have reported levels much lower than your proposed specification, we request that you lower your specification for *Enterobacteriaceae* to as low as can be reasonably obtained.

Additionally, you report the specification for aerobic plate count (APC) as <1,000 CFU/g and the specification for yeast and mold as <100 CFU/g. The results from the analytical testing reported in Figure 1 are at or below 20 CFU/g for APC and <10 CFU/g for yeast and mold. As you have reported levels much lower than your specifications, we request that you also consider lowering the specifications for APC, yeast, and mold to reflect the results from batch analyses.

We accept FDA's recommendation and have lowered the relevant finished product specifications as summarized below. Specifications in figures throughout this amendment reflect these new specifications.

- *Bacillus cereus*: <100 colony forming units (CFU)/g
- *Enterobacteriaceae*: <20 CFU/g
- APC: <100 CFU/g
- Yeast: <20 CFU/g
- Mold: <20 CFU/g

6: Information Requested

On page 6 of the July 28, 2023, amendment, in question 4, FDA asked you to provide an affirmative statement that you will test for the presence of toxic heavy metal contaminants in the harvested cell material on a more frequent ongoing basis, rather than "… at least twice in the next 12 months." In response to this question, you stated that testing for the presence of toxic heavy metal contaminants would occur "… on a more regular basis, rather than at least twice per year." Based on your response to this question, it is still unclear what your testing frequency for the presence of toxic heavy metal contaminants is. For addition to the disclosable safety narrative, please clarify whether "… on a more regular basis" means that you will test each batch of the harvested cell material. If this statement does not refer to testing each batch, please clarify the testing frequency and provide justification for why you believe the testing frequency provides an adequate level of public health protection.

We will test every batch of the harvested cell material as defined by FDA (cells harvested from bioreactors prior to freezing) for the presence of toxic heavy metal contaminants for a period of six months. Additional information on Wildtype's approach to testing is provided in our response to question 7 below.

7: Information Requested

On pages 6-7 of the July 28, 2023, amendment, in question 5, FDA asked you to provide an affirmative statement that you will test for the presence of all of the microorganisms identified as part of the "ongoing" analysis of the harvested cell material in Figure 5 of the January 17, 2023, amendment on a more frequent ongoing basis, rather than "… at least twice per year." In response to this question, you stated that testing for the presence of the microorganisms identified as part of the "ongoing" analysis of the harvested cell material listed in Figure 5 of the January 17, 2023, amendment would occur "… on a more regular basis, rather than at least twice per year."

On page 7 of the July 28, 2023, amendment, in response to question 6, you state "Wildtype will monitor or otherwise control for the presence of spores and toxins from microorganisms (e.g., *B. cereus*, *Staphylococcus aureus*) in the harvested cell material on a more frequent ongoing basis, rather than during a 90-day baseline period or "one time," respectively, as clarified in our May 3, 2023, amendment,

which may include adjustments in frequency over time based on production experience in different configurations."

Further, in response to question 5 of the July 28, 2023, amendment you state "Ongoing monitoring for spore formers such as *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum* has also been conducted on 2023 production lots;" however, Figure 1 on page 3 of the same amendment does not include *C. perfringens* or *C. botulinum*, only the toxins from those two microorganisms. In response to question 7 of the same amendment you state "The researchers at Texas Tech University who carried out the validation study identified one significant limitation to the study, namely the non-extensibility of this control to spore formers. Considering this limitation, other controls and measures are employed to limit spore formers including plant sanitation, personal protective equipment, GMPs, and testing of the finished product as described in our response to questions 2 and 6 above."

Based on your response to these questions, it is still unclear what your testing frequency for the presence of these microorganisms and toxins are. For addition to the disclosable safety narrative, please clarify the following:

a. whether "… on a more regular basis" means that you will test each batch of the harvested cell material. If this statement does not refer to testing each batch, please clarify the testing frequency;

b. whether the 3 microorganisms (*B. cereus, C. perfringens, and C. botulinum*) mentioned in your response to question 5 will be included in your "ongoing" analyses of the harvested cell material for the foreseeable future; and

c. whether testing for *C. perfringens* and *C. botulinum*, as reported in question 5 refers to the microorganisms, their toxins, or both.

If you are not proposing to test each batch, please provide justification for why you believe the testing frequency provides an adequate level of public health protection.

For the finished food products, we will follow the testing frequency listed below in Figure 4 for at least the first year of commercial production, at which time we will reassess this frequency based on a risk-based approach. Following the one year period, Wildtype will routinely test the finished product for all of the potential adventitious agents listed below at least quarterly to validate efficacy of controls. If this frequency is changed, we will submit a supplement to the completed consultation.

For the harvested cell material, we will follow the same testing frequency for the first six months of commercial production. After six months, if there is no material discrepancy between test results for the harvested cell material and test results for finished food products, then we would consider testing of the finished food products to be sufficient to detect contamination events that were present at the point of harvest. Following the six month period, Wildtype will routinely test the harvested cell material for all of the potential adventitious agents listed below at least quarterly to validate efficacy of controls. If this frequency is changed, we will submit a supplement to FDA.

Wildtype's GMP batch records assign a unique lot number to each batch of harvested cell material at the point of harvest from bioreactors. This unique lot number may be used to trace any contamination detected in the finished product to a specific bioreactor harvest. Finished food products are made using only one lot of cells, or a portion of one lot of cells, per lot of finished product, enabling clear traceability from bioreactor to finished product.

Figure 4 - testing frequency for harvested cell material and finished products

Potential Hazard	Frequency	Method[15]	Specification
Aerobic plate count	Every batch	AOAC OMA 990.12	<100 cfu/g
Yeast/mold	Every batch	AOAC OMA 2014.05	<20 cfu/g
Enterobacteriaceae	Every batch	AOAC OMA 2003.01 / USP 37 <61>	<20 cfu/g
Total coliforms	Every batch	AOAC OMA 991.14	<100 cfu/g
E. coli	Every batch	AOAC OMA 991.14	<20 cfu/g
***Campylobacter* species screen**	Every batch	AOAC RI 051201	Negative/25g
Salmonella	Every batch	AOAC OMA 2011.03	Negative/25g
***Listeria* genus**	Every batch	AOAC OMA 2013.10	Negative/25g
Staphylococcus aureus	Every batch	AOAC OMA 2003.07	<20 cfu/g
***Bacillus cereus* organism**	Every batch	FDA BAM	<100 cfu/g
***C. perfringens* organism**	Every batch	ISO 7937	<10 CFU/g
***C. botulinum* organism**	Every batch	FDA-BAM, 8th ed.	Negative/8g
Arsenic	Every batch	AOAC 2011.19, 993.14 and 2015.01	<100 / <50 ppb[16]
Cadmium	Every batch	AOAC 2011.19, 993.14 and 2015.01	<20 ppb
Mercury	Every batch	AOAC 2011.19, 993.14 and 2015.01	<50 ppb
Lead	Every batch	AOAC 2011.19, 993.14 and 2015.01	<20 ppb

Microorganism testing for *B. cereus, C. perfringens, and C. botulinum* was selected because the organisms are a necessary precursor to the associated toxins.[17] Additionally, testing for the organism may also detect the presence of vegetative spores.

Since commencing GMP operations in January 2023, Wildtype has produced and tested 32 batches of finished products produced using the same manufacturing process described in our July 28, 2023 amendment, all of which have been within specifications for potential adventitious agents.

8: Information Requested

On page 8 of the May 3, 2023, amendment, you state "... testing for cereulide is obviated by consistent testing that reveals the absence of *Bacillus cereus* contamination, as the bacterium is required for toxin production." For addition to the disclosable safety narrative, please provide a reference for this statement.

Yang et al. summarize the mechanisms of cereulide biosynthesis and the epidemiology of cereulide toxicity in a review published last year; in this review, they implicate *Bacillus cereus* alone as the source of this toxin.[18] Other reports, including that of Rouzeau-Szynalski et al., similarly implicate only *Bacillus cereus* in the production of cereulide, and exclusively focus cereulide food safety recommendations on the eradication of *Bacillus cereus*.[19]

Points of clarification

9: Information Requested

On page 2 of the July 28, 2023, amendment, in response to question 1, you state "The harvest process concludes with a validated thermal process (discussed at length in our January and May 2023

[15] All methods are validated for their intended purposes and are carried out by an external laboratory (e.g., Aemtek, Eurofins, Mérieuex).
[16] We have set the lowest possible arsenic specification for cells at the point of harvest to 100 ppb and have maintained our specification for finished products at 50 ppb. We have included adventitious agent testing data for three lots of finished products made using the cells in figure 1 in in appendix 4 (page 78). In all cases, arsenic and other heavy metals were below the limit of detection in the finished product.
[17] FDA BAM Chapter 17: *Clostridium botulinum: "If the organisms do not grow, no toxin is produced."*
[18] Yang S, Wang Y, Liu Y, Jia K, Zhang Z, Dong Q. Cereulide and Emetic *Bacillus cereus*: Characterizations, Impacts and Public Precautions. *Foods*. 2023 Feb 15;12(4):833. doi: 10.3390/foods12040833. PMID: 36832907; PMCID: PMC9956921.
[19] Rouzeau-Szynalski K, Stollewerk K, Messelhäusser U, Ehling-Schulz M. Why be serious about emetic *Bacillus cereus*: Cereulide production and industrial challenges. *Food Microbiol*. 2020 Feb;85:103279. doi: 10.1016/j.fm.2019.103279. Epub 2019 July 26. PMID: 31500702.

amendments) intended to render the cells non-viable and control for pathogens of concern such as *Listeria*, *Salmonella*, and others." On page 13 of the January 17, 2023, amendment, in response to question 8, you state that the thermal process occurs between 65-70°C. On page 24 of the same amendment, in response to question 19, you state "The product is then subjected to a number of processing steps as well as a terminal lethal step that ensures internal temperature reaches at least 70°C and remains at this temperature for at least 25 minutes." For addition to the disclosable safety narrative, please clarify the temperature and duration of the thermal process.

The thermal process is currently conducted at 70°C for a total of 110 minutes (including come-up time). This process significantly exceeds FDA's recommendation[20] to cook raw fish to an internal temperature of at least 145°F (63°C) for 15 seconds.

10: Information Requested

On page 2 of the July 28, 2023, amendment, in response to question 1, you state "Wildtype's scaffold inputs, which are introduced prior to termination of cell viability, have not changed from those disclosed in our January 17, 2023 amendment."

On page 14 of the disclosable safety narrative, you state "Inputs for Wildtype's scaffolds are gathered by operators, quality-checked, mixed, and assembled. Inputs are then sterilized using techniques such as heating or ethanol treatment, and are seeded with cells under aseptic conditions;" however, in response to question 19 in the January 17, 2023, amendment, you state "Although the cell proliferation stages occur under strictly aseptic conditions, the limited cell maturation stage and subsequent terminal lethal steps occurs without aseptic controls in an environment-controlled, high-care food production facility, where hygienic zoning and extra gowning requirements are present."

On page 2 of the July 28, 2023, amendment, you state "The most substantive change to steps 7 and 8 versus what was described in CCC 000005 is that the "maturation" stage, as it was described in CCC 000005 is currently excluded from Wildtype's process. The harvest process concludes with a validated thermal process (discussed at length in our January and May 2023 amendments) intended to render the cells non-viable and control for pathogens of concern such as Listeria, Salmonella, and others."

For addition to the disclosable safety narrative, given that there is no longer a "maturation" stage, please clarify the following:

a. The discrepancy regarding the aseptic conditions statement on page 14 of the disclosable safety narrative and the statement in response to question 19 of the January 17, 2023, amendment;

b. Whether the additional sterilization methodologies mentioned on page 14 of the disclosable safety narrative (i.e., ethanol treatment) are performed on the scaffold inputs. If so, please describe these methods and the frequency of performance. We note that only the thermal process is mentioned on page 11 of the July 28, 2023, amendment; and

c. Whether the sterilization methodologies mentioned on page 14 of the disclosable safety narrative control for the presence of Clostridium botulinum. We note that the thermal process described in the January 17, 2023, amendment would not be sufficient to control for the presence of proteolytic strains of C. botulinum.

Sterilization is not currently performed on the scaffold inputs.

[20] 2022 FDA Food Code Annex 7 -58, accessed using this link on 11/20/2023

Given that the cell maturation stage as described in CCC 000005 is currently excluded from our process, there is no need to sterilize scaffold inputs. The combination of cells and scaffold inputs occurs in an environment-controlled, high-care food production facility with strict hygienic zoning, under environmental monitoring program, and GMP requirements.

C. botulinum is controlled by minimizing cooling time via blast chillers and storing the finished product in a freezer at or below -17.8˚C (0ºF) with continuous monitoring. As described in our July 2023 amendment (appendix), refrigeration and freezer temperatures are monitored during the cooling and storage processes. This process is consistent with FDA guidance provided for controlling *C. botulinum* as described in the 2022 Food Code.[21] Additional details about our cooling and storage steps are included in our response to question 12 below.

11: Information Requested

On page 4 of the July 28, 2023, amendment, in response to question 3, you identify poloxamer 188 as a new media component. Poloxamer 188 is a polymer. On page 5 of the same amendment, Figure 3b, you list the evidence of use in food supply as "various." This statement pertaining to the safe use and current presence of poloxamer 188 in food is not supported by any references. We request that you provide references to support this statement, with complete citations.

Poloxamer 188 (9003-11-6 generic CASRN) is a non-ionic surfactant that is utilized to control shear forces in suspension cultures (ThermoFisher, accessed 06/2020). The safety of Poloxamer 188 was comprehensively reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (Singh-Joy et al., 2008 [22]) and is summarized herein. Other toxicity data associated with Poloxamer 188 and the generic CASRN 9003-11-6 were also considered.

Acute oral toxicity of Poloxamer 188 was demonstrated to be low in rats with an LD_{50} of 9380 mg/kg bw[23]. In humans, Poloxamer 188, which was approved by the FDA as a therapeutic reagent, was demonstrated to be safe when given for up to 72 hours and well tolerated upon repeated exposure in over the counter products[24].

Leaf[25] (as cited by Singh-Joy et al., 2008) conducted 6-month feeding studies in rats and dogs, whereby groups of 45 rats were administered 0, 3, or 5% Poloxamer 188 by weight in the diet, and four dogs/group received 0, 0.05, or 0.1 g/kg of Poloxamer 188 in capsule form prior to feeding. Overall results from both species did not reveal any toxicologically significant effects from dietary exposure to Poloxamer 188.

In two-year feeding studies of Poloxamer 188 administered in rats at doses of 0, 3, 5, and 7.5%, aside from moderate diarrhea observed at the two highest doses and minimally decreased growth at the highest tested dose without any effects on survival, no other adverse treatment-related effects were reported (Leaf, 1967, as cited by Singh-Joy et al., 2008).

In a drinking water study with high molecular weight poloxamer (11500 Da; 70% ethylene glycol), no adverse effects were reported in rats. The NOAEL was 15,000 ppm, corresponding to approximately 1,140

[21] 2022 FDA Food Code, Annex 3, 141-142 (Reduced Oxygen Packaging with One Barrier (Cook-Chill and Sous Vide). Accessed using this link on 28 November 2023.
[22] Singh-Joy SD, McLain VC. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. Int J Toxicol. 2008;27 Suppl 2:93-128. doi: 10.1080/10915810802244595. PMID: 18830866
[23] Drugbank (2021). Poloxamer 188. DB11333. Date accessed July 18, 2023 at https://go.drugbank.com/drugs/DB11333
[24] Moloughney JG and Weisleder N (2012). Poloxamer 188 (P188) as a Membrane Resealing Reagent in Biomedical Applications. Recent Pat Biotechnol, 6(3):200-211.
[25] Leaf, C. W.1967.Toxicology of some non-ionic surfactants.*Soap Chem. Spec.*43:48.

mg/kg bw/day in male and 1,560 mg/kg bw/day in female rats (EFSA, 2006). Toxicokinetic data in dogs indicate that there is substantial absorption from the gastrointestinal tract.[26]

Poloxamers with MW 3000-5000 Da demonstrated an equivocal potential for gene mutation induction in bacteria and mammalian cells in vitro, but no clastogenicity was observed. *In vivo* sister chromatid exchange assay in mammalian cells (Chinese hamster bone marrow cells) were negative. Based on the available *in vitro* and *in vivo* genotoxicity test data, including a poloxamer considered to be a worst case substance for toxicity, poloxamers demonstrate no genotoxic potential (EFSA, 2006).

The CIR Panel concluded that Poloxamers including Poloxamer 188 have a low order of toxicity. The available toxicological data do not suggest any concerns for carcinogenesis as well as significant exposure to reproductive organs or to the developing fetus. A NOAEL was not established for any of the studies reported in the CIR safety assessment (Singh-Joy et al., 2008).

Given that there are no safety concerns associated with this compound based on the available chronic oral toxicity study, including carcinogenesis, and the predicted exposure is infinitesimally small (4.62E-12 mg/kg bw/day), the presence of this compound in the media is not of safety concern.

We note that the use of Poloxamer 188 in the manufacture of a cell cultivated product was previously addressed in CCC 000001 (pages 54-58).

12: Information Requested

On page 20 of the July 28, 2023, amendment, you include information on activities performed on the refrigerator/freezer. On pages 23-24 of the January 17, 2023, amendment, in response to question 19, you state that, following the thermal processing step, "The product is then cooled in a refrigerator and stored at 4˚C for at least six hours before being stored for final shipment in a standard freezer (-20˚C)." For addition to the disclosable safety narrative, please clarify whether the information provided regarding the time and temperature of cooling and freezing accurately reflect your current production process.

Wildtype's finished products are cooled from 57.2°C to 21.1°C (135°F to 70°F) within two hours and from 57.2°C to 5°C (135°F to 41°F) within a total of six hours using a blast chiller. After the cooling process, products are stored for final shipment in a freezer at or below -17.8˚C (0°F). These cooling times are within FDA's guidelines as described in Fish and Fishery Products Hazards and Controls Guidance June 2022 Edition and 2022 Food Code page 624.

[26] European Food Safety Authority (EFSA) (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids, and material in contact with food (AFC) on a request related to an 11th list of substances for food contact materials. EFSA Journal (2006) 316 to 318; 1-10.

WILDTYPE

Appendix 1: Certificates of analysis for figures 1 & 2 of this amendment

eurofins | Microbiology

Eurofins Microbiology Laboratories (Los Angeles)

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	**Reference** AOAC 2011.19, 993.14 and 2015.01 (modified)	**Accreditation**	**Completed** 16Nov2023	**Sub** 5

Parameter	**Result**
Arsenic	0.0565 ppm
Cadmium	0.00583 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

JD00A - Clostridium perfringens Enterotoxin	**Reference** No Reference	**Completed** 17Nov2023

Parameter	**Result**
Enterotoxin Screen	Negative ng PET Type A ml

Subcontracting Partner: Toxin Technology, Inc. (Florida, USA)

QD038 - Carbohydrates, Calculated	**Reference** CFR 21-calc.	**Accreditation**	**Completed** 20Nov2023	**Sub** 2

Parameter	**Result**
Carbohydrates, Calculated	0.72 %

QD059 - Fat by Acid Hydrolysis	**Reference** AOAC 954.02	**Accreditation**	**Completed** 20Nov2023	**Sub** 2

Parameter	**Result**
Crude Fat By Acid Hydrolysis	1.82 %

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference** AOAC 996.06 mod.	**Accreditation**	**Completed** 20Nov2023	**Sub** 2

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		20Nov2023	2

Parameter	Result
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %
C12:0 (Lauric Acid)	<0.02 %
C14:0 (Myristic acid)	0.03 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	<0.02 %
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	0.15 %
C16:1 Omega 7	<0.04 %
C16:1 Total (Palmitoleic Acid + isomers)	0.07 %
C16:2 (Hexadecadienoic Acid)	<0.02 %
C16:3 (Hexadecatrienoic Acid)	<0.02 %
C 16:4 (Hexadecatetraenoic Acid)	<0.02 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	0.02 %
C18:0 (Stearic Acid)	0.09 %
C18:1 (Vaccenic acid)	<0.03 %
C18:1 Omega 9 (Oleic Acid)	0.38 %
C18:1, Total (Oleic Acid + isomers)	0.45 %
C18:2 Omega 6 (Linoleic Acid)	0.04 %
C18:2, Total (Linoleic Acid + isomers)	0.12 %
C18:3 Omega 3 (Alpha Linolenic Acid)	<0.02 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	<0.02 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	0.06 %
C18:4 Total (Octadecatetraenoic Acid)	0.06 %
C20:0 (Arachidic Acid)	<0.02 %
C20:1 Omega 9 (Gondoic Acid)	<0.02 %
C20:1 Total (Gondoic Acid + isomers)	0.02 %
C20:2 Omega 6	<0.02 %
C20:2 Total (Eicosadienoic Acid)	<0.02 %
C20:3 Omega 3	<0.02 %

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		20Nov2023	2

Parameter	Result
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %
C20:4 Omega 3	<0.02 %
C20:4 Omega 6 (Arachidonic Acid)	<0.02 %
C20:4, Total (Eicosatetraenoic Acid)	<0.02 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.02 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02 %
C22:0 (Behenic Acid)	<0.02 %
C22:1 Omega 9 (Erucic Acid)	<0.02 %
C22:1 Total (Erucic Acid + isomers)	<0.02 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	<0.02 %
C22:5 Docosapentaenoic Omega 6	<0.02 %
C22:5 Total (Docosapentaenoic Acid)	<0.02 %
C22:6 Docosahexaenoic Omega 3	0.03 %
C24:0 (Lignoceric Acid)	<0.02 %
C24:1 Omega 9 (Nervonic Acid)	0.02 %
C24:1 Total (Nervonic Acid + isomers)	0.03 %
Total Omega 3 Isomers	0.12 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	<0.05 %
Total Omega 7 Isomers	0.05 %
Total Omega 9 Isomers	0.49 %
Total Monounsaturated Fatty Acids	0.55 %
Total Polyunsaturated Fatty Acids	0.25 %
Total Saturated Fatty Acids	0.29 %
Total Trans Fatty Acids	0.06 %
Total Fat as Triglycerides	1.20 %
Total Fatty Acids	1.15 %

QD06X - Clostridium Botulinum Toxin - Presumptive	Reference	Accreditation	Completed	Sub
	No Reference		08Dec2023	3

Parameter	Result
Clostridium Botulinum Toxin	Negative per 50 g

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

	Reference	Accreditation	Completed	Sub
QD06X - Clostridium Botulinum Toxin - Presumptive	No Reference		08Dec2023	3

	Reference	Accreditation	Completed	Sub
QD148 - Moisture by Vacuum Oven	AOAC 925.09		20Nov2023	2

Parameter	Result
Moisture and Volatiles - Vacuum Oven	85.4 %

	Reference	Accreditation	Completed	Sub
QD226 - Calories, Calculated	CFR - Atwater calculation		20Nov2023	2

Parameter	Result
Calories Calculated	58 kcal/100 g

	Reference	Accreditation	Completed	Sub
QD250 - Ash	AOAC 942.05		20Nov2023	2

Parameter	Result
Ash	1.62 %

	Reference	Accreditation	Completed	Sub
QD252 - Protein - Combustion	AOAC 990.03; AOAC 992.15		20Nov2023	2

Parameter	Result
Protein	11.06 %
Nitrogen - Combustion	1.77 %
Protein Factor	6.25

	Reference	Accreditation	Completed	Sub
QD493 - Clostridium Botulinum Viable Cells - Presumptive	No Reference		08Dec2023	3

Parameter	Result
Clostridium botulinum (without toxin detection)	Negative per 8 g

	Reference	Accreditation	Completed
UM4BV - Yeast - FDA BAM Chapter 18 mod.	FDA BAM Chapter 18 mod.	ISO/IEC 17025:2017 A2LA 3329.05	15Nov2023

Parameter	Result
Yeast	< 10 cfu/g
Mold	< 10 cfu/g

	Reference	Accreditation	Completed	Sub
UM6NM - Campylobacter Species - AOAC RI #040702	AOAC-PTM 040702		13Nov2023	4

WILDTYPE

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ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

UM6NM - Campylobacter Species - AOAC RI #040702	**Reference** AOAC-PTM 040702	**Accreditation**	**Completed** 13Nov2023	**Sub** 4

Parameter	Result
Campylobacter Species	Not Detected per 25 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Nov2023

Parameter	Result
Total Coliforms	< 10 cfu/g
E. coli	< 10 cfu/g

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Nov2023

Parameter	Result
Escherichia coli O157:H7	Not Detected per 25 g

UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 12Nov2023

Parameter	Result
Staphylococcus aureus	< 10 cfu/g

UMJN3 - Non-O157 Shiga toxin-Producing E.coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 11Nov2023

Parameter	Result
Non-O157 Shiga toxin-Producing E.coli	Not Detected per 25 g

UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Nov2023

Parameter	Result
Enterobacteriaceae	10 (est) cfu/g

UMKXG - Staphylococcal Enterotoxin - AOAC 2007.06	**Reference** AOAC 2007.06	**Accreditation**	**Completed** 16Nov2023	**Sub** 1

Parameter	Result
Staphylococcal Enterotoxin	Not Detected per 25 g

WILDTYPE

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ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11100103	**Sample Registration Date:**	10Nov2023
Client Sample Code:	GMP salmon cells 1	**Condition Upon Receipt:**	acceptable, 3.5°C
Sample Description:	GMP salmon cells	**Sample Reference:**	

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 12Nov2023

Parameter	**Result**
Bacillus cereus	< 10 cfu/g

UMQE5 - Listeria monocytogenes - AOAC-RI 061703	**Reference** AOAC-RI 061703	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Nov2023

Parameter	**Result**
Listeria monocytogenes	Not Detected per 25 g

UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Nov2023

Parameter	**Result**
Salmonella spp.	Not Detected per 25 g

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 12Nov2023

Parameter	**Result**
Aerobic Plate Count	< 10 cfu/g

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Des Moines), IA
2 - Eurofins Nutrition Analysis Center, Iowa
3 - Silliker, INC Food Science Center, IL
4 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
5 - Eurofins Food Chemistry Testing US Madison, Wisconsin

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager

WILDTYPE

Wild Type, Inc.

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ANALYTICAL REPORT

AR-23-QR-037088-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1 - Nov 2023

Received On: 10Nov2023
Reported On: 08Dec2023

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Eurofins Microbiology Laboratories (Los Angeles)

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-036143-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1-Nov 2023

Received On: 16Nov2023
Reported On: 27Nov2023

Eurofins Sample Code: 111-2023-11160168
Client Sample Code: 110923
Sample Description: GMP salmon cells 1
Sample Registration Date: 16Nov2023
Condition Upon Receipt: acceptable, 6.8°C
Sample Reference:

QD0EK - Vitamin D (LC-MS/MS)	Reference	Accreditation	Completed	Sub
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		27Nov2023	1

Parameter	Result
Total Vitamin D2 and D3	6,760 IU/100 g
Vitamin D2	6,730 IU/100 g
Vitamin D3	33.3 IU/100 g

QQ151 - Total Vitamin B12-Cobalamin(Low Level <3 mg/100g)	Reference	Accreditation	Completed	Sub
	AOAC 952.20 mod.		27Nov2023	1

Parameter	Result
Vitamin B12	248 µg/100 g

QQ156 - Total Vitamin B5-Pan Acid(Low Level <100 mg/100g)	Reference	Accreditation	Completed	Sub
	AOAC 945.74 (mod.)		27Nov2023	1

Parameter	Result
Pantothenic acid	1.35 mg/100 g

QQ182 - Total Vitamin A	Reference	Accreditation	Completed	Sub
	AOAC 974.29 Mod.		27Nov2023	1

Parameter	Result
ß-carotene	<30 IU/100 g
Retinol	<30 IU/100 g
Total Vitamin A	<30 IU/100 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	Reference	Accreditation	Completed
	CMMEF Chapter 9.933	ISO/IEC 17025:2017 A2LA 3329.05	17Nov2023

Parameter	Result
Total Coliforms	< 10 cfu/g

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ANALYTICAL REPORT

AR-23-QR-036143-01

Client Code: QR0000417
PO#: FDA RFI - cell test 1-Nov 2023

Received On: 16Nov2023
Reported On: 27Nov2023

Eurofins Sample Code:	111-2023-11160168	**Sample Registration Date:**	16Nov2023
Client Sample Code:	110923	**Condition Upon Receipt:**	acceptable, 6.8°C
Sample Description:	GMP salmon cells 1	**Sample Reference:**	

UM8VD - E. coli - CMMEF Chapter 9.933	**Reference**	**Accreditation**	**Completed**
	CMMEF Chapter 9.933	ISO/IEC 17025:2017 A2LA 3329.05	17Nov2023

Parameter	**Result**
E. coli	< 10 cfu/g

Subcontracting partners:
1 - Eurofins Nutrition Analysis Center, Iowa

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager

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Eurofins Microbiology Laboratories (Los Angeles)

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Wild Type, Inc.

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ANALYTICAL REPORT

AR-23-QR-035450-01

Client Code: QR0000417
PO#: FDA RFI-cell test 1- Nov 2023

Received On: 16Nov2023
Reported On: 17Nov2023

Eurofins Sample Code:	111-2023-11160166	**Sample Registration Date:**	16Nov2023
Client Sample Code:	110323	**Condition Upon Receipt:**	acceptable, 6.8°C
Sample Description:	GMP salmon cells 1	**Sample Reference:**	

ZM3KF - Clostridium perfringens - ISO 7937	**Reference** ISO 7937	**Completed** 17Nov2023

Parameter	**Result**
Clostridium perfringens	< 10 cfu/g

Respectfully Submitted,

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Viridiana Castro
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ANALYTICAL REPORT

AR-23-QR-035879-01

Client Code: QR0000417
PO#: For FDA RFI - Nov 2023

Received On: 16Nov2023
Reported On: 23Nov2023

Eurofins Sample Code:	111-2023-11160167	**Sample Registration Date:**	16Nov2023
Client Sample Code:	110323	**Condition Upon Receipt:**	acceptable, 6.8°C
Sample Description:	GMP salmon cells 1	**Sample Reference:**	

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	Reference	Accreditation	Completed	Sub
	AOAC 992.05 mod.		22Nov2023	1

Parameter	Result
Total Folate as Folic Acid	0.0780 mg/100 g

Subcontracting partners:
1 - Eurofins Nutrition Analysis Center, Iowa

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 2011.19, 993.14 and 2015.01 (modified)		27Nov2023	5

Parameter	**Result**
Arsenic	0.0810 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

QD038 - Carbohydrates, Calculated	**Reference**	**Accreditation**	**Completed**	**Sub**
	CFR 21-calc.		07Dec2023	2

Parameter	**Result**
Carbohydrates, Calculated	<0.50 %

QD059 - Fat by Acid Hydrolysis	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 954.02		07Dec2023	2

Parameter	**Result**
Crude Fat By Acid Hydrolysis	1.71 %

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 996.06 mod.		07Dec2023	2

Parameter	**Result**
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %
C12:0 (Lauric Acid)	<0.02 %

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ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		07Dec2023	2

Parameter	Result
C14:0 (Myristic acid)	0.03 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	<0.02 %
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	0.14 %
C16:1 Omega 7	<0.04 %
C16:1 Total (Palmitoleic Acid + isomers)	0.06 %
C16:2 (Hexadecadienoic Acid)	<0.02 %
C16:3 (Hexadecatrienoic Acid)	<0.02 %
C 16:4 (Hexadecatetraenoic Acid)	<0.02 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	0.02 %
C18:0 (Stearic Acid)	0.06 %
C18:1 (Vaccenic acid)	<0.03 %
C18:1 Omega 9 (Oleic Acid)	0.36 %
C18:1, Total (Oleic Acid + isomers)	0.41 %
C18:2 Omega 6 (Linoleic Acid)	<0.02 %
C18:2, Total (Linoleic Acid + isomers)	0.10 %
C18:3 Omega 3 (Alpha Linolenic Acid)	<0.02 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	<0.02 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02 %
C18:4 Total (Octadecatetraenoic Acid)	<0.02 %
C20:0 (Arachidic Acid)	<0.02 %
C20:1 Omega 9 (Gondoic Acid)	<0.02 %
C20:1 Total (Gondoic Acid + isomers)	<0.02 %
C20:2 Omega 6	<0.02 %
C20:2 Total (Eicosadienoic Acid)	<0.02 %
C20:3 Omega 3	<0.02 %
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %
C20:4 Omega 3	<0.02 %
C20:4 Omega 6 (Arachidonic Acid)	<0.02 %
C20:4, Total (Eicosatetraenoic Acid)	<0.02 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	<0.02 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02 %

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ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		07Dec2023	2

Parameter	Result
C22:0 (Behenic Acid)	<0.02 %
C22:1 Omega 9 (Erucic Acid)	<0.02 %
C22:1 Total (Erucic Acid + isomers)	<0.02 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	<0.02 %
C22:5 Docosapentaenoic Omega 6	<0.02 %
C22:5 Total (Docosapentaenoic Acid)	<0.02 %
C22:6 Docosahexaenoic Omega 3	<0.02 %
C24:0 (Lignoceric Acid)	<0.02 %
C24:1 Omega 9 (Nervonic Acid)	<0.02 %
C24:1 Total (Nervonic Acid + isomers)	<0.02 %
Total Omega 3 Isomers	<0.05 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	<0.05 %
Total Omega 7 Isomers	<0.05 %
Total Omega 9 Isomers	0.46 %
Total Monounsaturated Fatty Acids	0.51 %
Total Polyunsaturated Fatty Acids	0.12 %
Total Saturated Fatty Acids	0.24 %
Total Trans Fatty Acids	0.04 %
Total Fat as Triglycerides	0.95 %
Total Fatty Acids	0.91 %

QD06X - Clostridium Botulinum Toxin - Presumptive	Reference	Accreditation	Completed	Sub
	No Reference		08Dec2023	3

Parameter	Result
Clostridium Botulinum Toxin	Negative per 50 g

QD0EK - Vitamin D (LC-MS/MS)	Reference	Accreditation	Completed	Sub
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		07Dec2023	2

Parameter	Result
Total Vitamin D2 and D3	9,790 IU/100 g
Vitamin D2	9,790 IU/100 g

WILDTYPE

Wild Type, Inc.
[redacted]

ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code: 111-2023-11180039
Client Sample Code: 111623
Sample Description: GMP salmon cells 2
Sample Registration Date: 18Nov2023
Condition Upon Receipt: acceptable, -17.6°C
Sample Reference:

QD0EK - Vitamin D (LC-MS/MS)	Reference	Accreditation	Completed	Sub
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		07Dec2023	2

Parameter	Result
Vitamin D3	<4 IU/100 g

QD148 - Moisture by Vacuum Oven	Reference	Accreditation	Completed	Sub
	AOAC 925.09		07Dec2023	2

Parameter	Result
Moisture and Volatiles - Vacuum Oven	87.1 %

QD226 - Calories, Calculated	Reference	Accreditation	Completed	Sub
	CFR - Atwater calculation		07Dec2023	2

Parameter	Result
Calories Calculated	49 kcal/100 g

QD250 - Ash	Reference	Accreditation	Completed	Sub
	AOAC 942.05		07Dec2023	2

Parameter	Result
Ash	1.93 %

QD252 - Protein - Combustion	Reference	Accreditation	Completed	Sub
	AOAC 990.03; AOAC 992.15		07Dec2023	2

Parameter	Result
Protein	10.19 %
Nitrogen - Combustion	1.63 %
Protein Factor	6.25

QD493 - Clostridium Botulinum Viable Cells - Presumptive	Reference	Accreditation	Completed	Sub
	No Reference		08Dec2023	3

Parameter	Result
Clostridium botulinum (without toxin detection)	Negative per 8 g

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	Reference	Accreditation	Completed	Sub
	AOAC 992.05 mod.		07Dec2023	2

Parameter	Result
Total Folate as Folic Acid	0.0915 mg/100 g

WILDTYPE

Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

QQ151 - Total Vitamin B12-Cobalamin(Low Level <3 mg/100g)	**Reference** AOAC 952.20 mod.	**Accreditation**	**Completed** 07Dec2023	**Sub** 2

Parameter	**Result**
Vitamin B12	201 µg/100 g

QQ156 - Total Vitamin B5-Pan Acid(Low Level <100 mg/100g)	**Reference** AOAC 945.74 (mod.)	**Accreditation**	**Completed** 07Dec2023	**Sub** 2

Parameter	**Result**
Pantothenic acid	1.80 mg/100 g

QQ182 - Total Vitamin A	**Reference** AOAC 974.29 Mod.	**Accreditation**	**Completed** 07Dec2023	**Sub** 2

Parameter	**Result**
ß-carotene	<30 IU/100 g
Retinol	<30 IU/100 g
Total Vitamin A	<30 IU/100 g

UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 23Nov2023

Parameter	**Result**
Yeast	< 10 cfu/g
Mold	< 10 cfu/g

UM6NM - Campylobacter Species - AOAC RI #040702	**Reference** AOAC-PTM 040702	**Accreditation**	**Completed** 24Nov2023	**Sub** 4

Parameter	**Result**
Campylobacter Species	Not Detected per 25 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**
Total Coliforms	< 10 cfu/g
E. coli	< 10 cfu/g

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**

WILDTYPE

Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**
Escherichia coli O157:H7	Not Detected per 25 g

UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 20Nov2023

Parameter	**Result**
Staphylococcus aureus	< 10 cfu/g

UMJN3 - Non-O157 Shiga toxin-Producing E.coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 19Nov2023

Parameter	**Result**
Non-O157 Shiga toxin-Producing E.coli	Not Detected per 25 g

UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**
Enterobacteriaceae	< 10 cfu/g

UMKXG - Staphylococcal Enterotoxin - AOAC 2007.06	**Reference** AOAC 2007.06	**Accreditation**	**Completed** 23Nov2023	**Sub** 1

Parameter	**Result**
Staphylococcal Enterotoxin	Not Detected per 25 g

UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 20Nov2023

Parameter	**Result**
Bacillus cereus	< 10 cfu/g

UMQE5 - Listeria monocytogenes - AOAC-RI 061703	**Reference** AOAC-RI 061703	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**
Listeria monocytogenes	Not Detected per 25 g

WILDTYPE

Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-23-QR-037089-01

Client Code: QR0000417
PO#: FDA RFI - cell test 2 - Nov 2023

Received On: 18Nov2023
Reported On: 08Dec2023

Eurofins Sample Code:	111-2023-11180039	**Sample Registration Date:**	18Nov2023
Client Sample Code:	111623	**Condition Upon Receipt:**	acceptable, -17.6°C
Sample Description:	GMP salmon cells 2	**Sample Reference:**	

UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 19Nov2023

Parameter	**Result**
Salmonella spp.	Not Detected per 25 g

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 20Nov2023

Parameter	**Result**
Aerobic Plate Count	< 10 cfu/g

ZM3KF - Clostridium perfringens - ISO 7937	**Reference** ISO 7937	**Completed** 19Nov2023

Parameter	**Result**
Clostridium perfringens	< 10 cfu/g

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Des Moines), IA
2 - Eurofins Nutrition Analysis Center, Iowa
3 - Silliker, INC Food Science Center, IL
4 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
5 - Eurofins Food Chemistry Testing US Madison, Wisconsin

Respectfully Submitted,

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Wild Type, Inc.

[redacted]

ANALYTICAL REPORT

AR-24-QR-000198-01

Client Code: QR0000417
PO#: FDA RFI - cell test 4 - Nov 2023

Received On: 06Dec2023
Reported On: 02Jan2024

Eurofins Sample Code:	111-2023-12060075	**Sample Registration Date:**	06Dec2023
Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

FS001 - Heavy Metals (As, Cd, Hg, and Pb)	Reference	Accreditation	Completed	Sub
	AOAC 2011.19, 993.14 and 2015.01 (modified)		13Dec2023	5

Parameter	Result
Arsenic	0.0975 ppm
Cadmium	<0.00500 ppm
Lead	<0.00500 ppm
Mercury	<0.00500 ppm

FS011 - Inorganic Arsenic	Reference	Accreditation	Completed	Sub
	FDA Sect 4.11; ver 1.1 (2012); Thermo Sc note 430		28Dec2023	5

Parameter	Result
Sum of As from Inorganic Species	<0.0388 ppm

QD038 - Carbohydrates, Calculated	Reference	Accreditation	Completed	Sub
	CFR 21-calc.		20Dec2023	2

Parameter	Result
Carbohydrates, Calculated	<0.50 %

QD059 - Fat by Acid Hydrolysis	Reference	Accreditation	Completed	Sub
	AOAC 954.02		20Dec2023	2

Parameter	Result
Crude Fat By Acid Hydrolysis	1.54 %

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		20Dec2023	2

Parameter	Result
Fatty Acid Profile	Reported as Fatty Acids
C4:0 (Butyric Acid)	<0.02 %

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ANALYTICAL REPORT

AR-24-QR-000198-01

Client Code: QR0000417
PO#: FDA RFI - cell test 4 - Nov 2023

Received On: 06Dec2023
Reported On: 02Jan2024

Eurofins Sample Code:	111-2023-12060075	**Sample Registration Date:**	06Dec2023
Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	Reference	Accreditation	Completed	Sub
	AOAC 996.06 mod.		20Dec2023	2

Parameter	Result
C6:0 (Caproic acid)	<0.02 %
C8:0 (Caprylic acid)	<0.02 %
C10:0 (Capric acid)	<0.02 %
C11:0 (Undecanoic acid)	<0.02 %
C12:0 (Lauric Acid)	<0.02 %
C14:0 (Myristic acid)	0.04 %
C14:1 (Myristoleic acid)	<0.02 %
C15:0 (Pentadecanoic acid)	<0.02 %
C15:1 (Pentadecenoic acid)	<0.02 %
C16:0 (Palmitic Acid)	0.15 %
C16:1 Omega 7	<0.04 %
C16:1 Total (Palmitoleic Acid + isomers)	0.07 %
C16:2 (Hexadecadienoic Acid)	<0.02 %
C16:3 (Hexadecatrienoic Acid)	<0.02 %
C 16:4 (Hexadecatetraenoic Acid)	<0.02 %
C17:0 (Margaric Acid)	<0.02 %
C17:1 (Heptadecenoic Acid)	<0.02 %
C18:0 (Stearic Acid)	0.07 %
C18:1 (Vaccenic acid)	<0.03 %
C18:1 Omega 9 (Oleic Acid)	0.38 %
C18:1, Total (Oleic Acid + isomers)	0.43 %
C18:2 Omega 6 (Linoleic Acid)	<0.02 %
C18:2, Total (Linoleic Acid + isomers)	0.11 %
C18:3 Omega 3 (Alpha Linolenic Acid)	<0.02 %
C18:3 Omega 6 (Gamma Linolenic Acid)	<0.02 %
C18:3, Total (Linolenic Acid + isomers)	<0.02 %
C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02 %
C18:4 Total (Octadecatetraenoic Acid)	<0.02 %
C20:0 (Arachidic Acid)	<0.02 %
C20:1 Omega 9 (Gondoic Acid)	<0.02 %
C20:1 Total (Gondoic Acid + isomers)	0.02 %
C20:2 Omega 6	<0.02 %
C20:2 Total (Eicosadienoic Acid)	<0.02 %
C20:3 Omega 3	<0.02 %
C20:3 Omega 6	<0.02 %
C20:3, Total (Eicosatrienoic Acid)	<0.02 %

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ANALYTICAL REPORT

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Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

QD05C - Fatty Acids-Full Omega 9,6&3 & Trans %W/W	**Reference**	**Accreditation**	**Completed**	**Sub**
	AOAC 996.06 mod.		20Dec2023	2

Parameter	**Result**
C20:4 Omega 3	<0.02 %
C20:4 Omega 6 (Arachidonic Acid)	<0.02 %
C20:4, Total (Eicosatetraenoic Acid)	<0.02 %
C20:5 Omega 3 (Eicosapentaenoic Acid)	<0.02 %
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02 %
C22:0 (Behenic Acid)	<0.02 %
C22:1 Omega 9 (Erucic Acid)	<0.02 %
C22:1 Total (Erucic Acid + isomers)	<0.02 %
C22:2 Docosadienoic Omega 6	<0.02 %
C22:3 Docosatrienoic, Omega 3	<0.02 %
C22:4 Docosatetraenoic Omega 6	<0.02 %
C22:5 Docosapentaenoic Omega 3	<0.02 %
C22:5 Docosapentaenoic Omega 6	<0.02 %
C22:5 Total (Docosapentaenoic Acid)	<0.02 %
C22:6 Docosahexaenoic Omega 3	<0.02 %
C24:0 (Lignoceric Acid)	<0.02 %
C24:1 Omega 9 (Nervonic Acid)	<0.02 %
C24:1 Total (Nervonic Acid + isomers)	<0.02 %
Total Omega 3 Isomers	<0.05 %
Total Omega 5 Isomers	<0.05 %
Total Omega 6 Isomers	<0.05 %
Total Omega 7 Isomers	0.05 %
Total Omega 9 Isomers	0.49 %
Total Monounsaturated Fatty Acids	0.52 %
Total Polyunsaturated Fatty Acids	0.13 %
Total Saturated Fatty Acids	0.27 %
Total Trans Fatty Acids	0.04 %
Total Fat as Triglycerides	1.00 %
Total Fatty Acids	0.96 %

QD06X - Clostridium Botulinum Toxin - Presumptive	**Reference**	**Accreditation**	**Completed**	**Sub**
	No Reference		02Jan2024	3

Parameter	**Result**
Clostridium Botulinum Toxin	Negative per 50 g

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ANALYTICAL REPORT

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Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

QD0EK - Vitamin D (LC-MS/MS)	Reference	Accreditation	Completed	Sub
	Huang et al., Rapid Commun. Mass Spectrum 2014, 28		20Dec2023	2

Parameter	Result
Total Vitamin D2 and D3	9,210 IU/100 g
Vitamin D2	9,210 IU/100 g
Vitamin D3	<20 IU/100 g

QD148 - Moisture by Vacuum Oven	Reference	Accreditation	Completed	Sub
	AOAC 925.09		20Dec2023	2

Parameter	Result
Moisture and Volatiles - Vacuum Oven	86.0 %

QD226 - Calories, Calculated	Reference	Accreditation	Completed	Sub
	CFR - Atwater calculation		20Dec2023	2

Parameter	Result
Calories Calculated	53 kcal/100 g

QD250 - Ash	Reference	Accreditation	Completed	Sub
	AOAC 942.05		20Dec2023	2

Parameter	Result
Ash	2.05 %

QD252 - Protein - Combustion	Reference	Accreditation	Completed	Sub
	AOAC 990.03; AOAC 992.15		20Dec2023	2

Parameter	Result
Protein	10.69 %
Nitrogen - Combustion	1.71 %
Protein Factor	6.25

QD493 - Clostridium Botulinum Viable Cells - Presumptive	Reference	Accreditation	Completed	Sub
	No Reference		02Jan2024	3

Parameter	Result
Clostridium botulinum (without toxin detection)	Negative per 8 g

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	Reference	Accreditation	Completed	Sub
	AOAC 992.05 mod.		20Dec2023	2

Parameter	Result

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ANALYTICAL REPORT

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Eurofins Sample Code:	111-2023-12060075	**Sample Registration Date:**	06Dec2023
Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

QQ059 - Total Vitamin B9-Folate(Low Level <12.5 mg/100g)mg	**Reference** AOAC 992.05 mod.	**Accreditation**	**Completed** 20Dec2023	**Sub** 2

Parameter	**Result**
Total Folate as Folic Acid	0.0441 mg/100 g

QQ151 - Total Vitamin B12-Cobalamin(Low Level <3 mg/100g)	**Reference** AOAC 952.20 mod.	**Accreditation**	**Completed** 20Dec2023	**Sub** 2

Parameter	**Result**
Vitamin B12	230 µg/100 g

QQ156 - Total Vitamin B5-Pan Acid(Low Level <100 mg/100g)	**Reference** AOAC 945.74 (mod.)	**Accreditation**	**Completed** 20Dec2023	**Sub** 2

Parameter	**Result**
Pantothenic acid	1.28 mg/100 g

QQ182 - Total Vitamin A	**Reference** AOAC 974.29 Mod.	**Accreditation**	**Completed** 20Dec2023	**Sub** 2

Parameter	**Result**
ß-carotene	<30 IU/100 g
Retinol	<30 IU/100 g
Total Vitamin A	<30 IU/100 g

UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 11Dec2023

Parameter	**Result**
Yeast	< 10 cfu/g
Mold	< 10 cfu/g

UM6NM - Campylobacter Species - AOAC RI #040702	**Reference** AOAC-PTM 040702	**Accreditation**	**Completed** 12Dec2023	**Sub** 4

Parameter	**Result**
Campylobacter Species	Not Detected per 25 g

UM8VD - Total Coliforms - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 07Dec2023

Parameter	**Result**
Total Coliforms	< 10 cfu/g

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ANALYTICAL REPORT

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Reported On: 02Jan2024

Eurofins Sample Code:	111-2023-12060075	**Sample Registration Date:**	06Dec2023
Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

UM8VD - E. coli - CMMEF Chapter 9.933	**Reference** CMMEF Chapter 9.933	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 07Dec2023	
Parameter	**Result**			
E. coli	< 10 cfu/g			
UMEWE - Escherichia Coli O157:H7 - AOAC-RI 031002	**Reference** AOAC-RI 031002	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 07Dec2023	
Parameter	**Result**			
Escherichia coli O157:H7	Not Detected per 25 g			
UMHBM - Staphylococcus aureus - BAM Chapter 12	**Reference** BAM Chapter 12	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 08Dec2023	
Parameter	**Result**			
Staphylococcus aureus	< 10 cfu/g			
UMJN3 - Non-O157 Shiga toxin-Producing E.coli - AOAC-RI 091301	**Reference** AOAC-RI 091301		**Completed** 07Dec2023	
Parameter	**Result**			
Non-O157 Shiga toxin-Producing E.coli	Not Detected per 25 g			
UMKTF - Enterobacteriaceae - CMMEF Chapter 9.62	**Reference** CMMEF Chapter 9.62	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 07Dec2023	
Parameter	**Result**			
Enterobacteriaceae	< 10 cfu/g			
UMKXG - Staphylococcal Enterotoxin - AOAC 2007.06	**Reference** AOAC 2007.06	**Accreditation**	**Completed** 14Dec2023	**Sub** 1
Parameter	**Result**			
Staphylococcal Enterotoxin	Not Detected per 25 g			
UMMA7 - Bacillus cereus - BAM Chapter 14	**Reference** FDA BAM Chapter 14	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 08Dec2023	
Parameter	**Result**			
Bacillus cereus	< 10 cfu/g			

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ANALYTICAL REPORT

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Eurofins Sample Code:	111-2023-12060075	**Sample Registration Date:**	06Dec2023
Client Sample Code:	GMP salmon cells 4-fresh	**Condition Upon Receipt:**	atypical, 17.6°C
Sample Description:	GMP salmon cells 4-fresh	**Sample Reference:**	Harvested on 2023-11-30

UMQE5 - Listeria monocytogenes - AOAC-RI 061703	**Reference** AOAC-RI 061703	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 08Dec2023

Parameter	**Result**
Listeria monocytogenes	Not Detected per 25 g

UMQMM - Salmonella species - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 08Dec2023

Parameter	**Result**
Salmonella spp.	Not Detected per 25 g

UMVEP - Aerobic Plate Count - AOAC 966.23	**Reference** AOAC 966.23	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.05	**Completed** 08Dec2023

Parameter	**Result**
Aerobic Plate Count	< 10 cfu/g

ZM3KF - Clostridium perfringens - ISO 7937	**Reference** ISO 7937	**Completed** 07Dec2023

Parameter	**Result**
Clostridium perfringens	< 10 cfu/g

Received Condition: results may be adversely affected by samples' temperature

Subcontracting partners:

1 - Eurofins Microbiology Laboratories (Des Moines), IA
2 - Eurofins Nutrition Analysis Center, Iowa
3 - Silliker, INC Food Science Center, IL
4 - Eurofins Microbiology Laboratories (Lancaster), Pennsylvania
5 - Eurofins Food Chemistry Testing US Madison, Wisconsin

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ANALYTICAL REPORT

AR-24-QR-000198-01

Client Code: QR0000417
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Received On: 06Dec2023
Reported On: 02Jan2024

Respectfully Submitted,

[redacted]

Viridiana Castro
Business Unit Manager