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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

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# Food safety considerations and research priorities for the cultured meat and seafood industry

**Kimberly J. Ong**[1] | **Jeremiah Johnston**[2] | **Isha Datar**[2] | **Vincent Sewalt**[3] | **Dwayne Holmes**[4] | **Jo Anne Shatkin**[1]

[1] Vireo Advisors, LLC, Boston, Massachusetts, USA

[2] New Harvest Inc., Cambridge, Massachusetts, USA

[3] IFF, Palo Alto, California, USA

[4] Mosa Meat, Maastricht, The Netherlands

**Correspondence**
Kimberly J. Ong, Vireo Advisors, LLC, 111 Perkins Street, # 223, Boston, MA 02130, USA.
Email: kong@vireoadvisors.com

## Abstract

Cell-cultured meat and seafood offer a sustainable opportunity to meet the world's increasing demand for protein in a climate-changed world. A responsible, data-driven approach to assess and demonstrate safety of cell-cultured meat and seafood can support consumer acceptance and help fully realize the potential of these products. As an initial step toward a thorough demonstration of safety, this review identifies hazards that could be introduced during manufacturing, evaluates applicability of existing safety assessment approaches, and highlights research priorities that could support safe commercialization. Input was gathered from members of the cultured meat and seafood industry, researchers, regulators, and food safety experts. A series of workshops were held with 87 industry representatives and researchers to create a modular manufacturing process diagram, which served as a framework to identify potential chemical and biological hazards along the steps of the manufacturing process that could affect the safety of a final food product. Interviews and feedback on draft documents validated the process diagram and supported hazard identification and evaluation of applicable safety methods. Most hazards are not expected to be novel; therefore, safety assessment methods from a range of fields, such as conventional and novel foods, foods produced from biotechnology, pharmaceuticals, and so forth, are likely to be applicable. However, additional assessment of novel inputs or products with significant differences from existing foods may be necessary. Further research on the safety of the inputs and associated residues, potential for contamination, and development of standardized safety assessment approaches (particularly animal-free methods) is recommended.

**KEYWORDS**
cultured meat and seafood, hazard, methods, risk assessment, safety

# 1 | INTRODUCTION

Globally, the demand for meat and other animal-based products is expected to increase dramatically as the world's population grows and the socioeconomic status of developing countries inflates. By 2050, the human population is expected to swell to 9.5 billion, and the increase in demand for animal-based protein is projected to double (FAO, 2019; Henchion et al., 2017). Reliance on traditional, animal-based production is a highly inefficient way of meeting this increased demand (Alexander et al., 2017). Today, industrial animal agriculture is a significant source of environmental stress and raises concerns regarding sustainability, food safety and security, worker safety, public health, and the ethical treatment of animals. Cell-cultured meat and seafood production has the potential to provide a significant supply of animal protein and can help enhance global food security while offering human health and environmental and animal welfare benefits. Public acceptance and successful commercialization will require addressing ethical, environmental, and human health issues, along with consumer perception and aspects such as taste and affordability (Gaydhane et al., 2018). Fully realizing the potential of cell-cultured products demands a responsible approach to food safety to warrant regulatory, investor, and public acceptance (Ketelings et al., 2021). Safety demonstration, in turn, requires understanding the process of cell-cultured meat and seafood manufacture to identify potential hazards and food safety requirements. Consolidating this knowledge paves the way to identify and mitigate possible concerns while identifying opportunities to implement best practices.

To address cell-cultured product safety, a common understanding of the manufacturing process is needed (Ketelings et al., 2021). As part of this review, a generalized manufacturing process diagram was developed that portrays the majority of cell-cultured meat and seafood production. This diagram serves as a framework to identify potential manufacturing hazards, and was developed in consultation with 87 industry representatives from 50 cell-cultured meat and seafood companies and cell-cultured meat and seafood researchers, who substantiated the accuracy and validity of the diagram. Each process step has been assessed to identify potential biological and chemical hazards that could affect the safety of the final food product or cause human health effects. Some hazards are common throughout the process (e.g., the potential for contamination), whereas others are specific to a processing step (e.g., the use of cryoprotectants during cell storage). Physical hazards, although important to safety assessment, are not deemed to be unique to cell-cultured meats produced under the quality standards applied to all foods and are therefore not within the scope of this review. This review is not a risk assessment, as exposure to each of the hazards is likely to be process specific, but is intended to support such an assessment. Some principles, standards, and methods from developed disciplines such as conventional food and feed, novel foods, products of modern biotechnology, medicines, and other biological products will be applicable for risk assessment of cell-cultured products.

This review initiates the evaluation of cell-cultured meat and seafood manufacturing processes from a risk assessment perspective by first identifying the universe of potential hazards during product development, and then reviews where existing methodological approaches to safety assessment may be relevant for cell-cultured meat and seafood products. From this, current knowledge gaps are identified regarding the safety of cell-cultured meat and seafood products requiring further research. The overall purpose is to support researchers, product developers, manufacturers, and regulators as they identify potential hazards that may be encountered during the manufacturing process that could affect the safety of a final cell-cultured meat or seafood product.

## 1.1 | Defining cell-cultured meat and seafood

Cellular agriculture is broadly defined as the production of meat, milk, eggs, seafood, and other products and ingredients, from cell cultures rather than from farmed animals. Products of cellular agriculture can be classified along multiple dimensions. One dimension focuses on the type of product created—either cellular (i.e., composed of living or once-living cells) or acellular (i.e., composed of proteins, lipids, or other small molecules). Cellular agriculture can also be grouped by production method: either tissue engineering based or fermentation based (Stephens et al., 2018). This review focuses specifically on cellular products made from animal cells via tissue engineering processes as upcoming market and safety issues have not yet been well studied.

Multiple terms are in use for meat and seafood produced by in vitro techniques, including clean meat, in vitro meat, cell-based meat, cultured meat, cultivated meat, and more. The term used by the authors (*cell-cultured meat and seafood*) focuses on the process by which the meat and seafood was created (i.e., cell culture) rather than focusing on the product (i.e., cell-based) or any theoretical qualities of the product (i.e., clean).

Cell-cultured meat and seafood processes are largely based on tissue engineering principles developed for biomedical applications (Stephens et al., 2018). In tissue engineering–based systems, animal cells are harvested from living animals, developed into cell lines, and

engineered to manufacture the desired product. Products may incorporate cell-cultured meat as an ingredient, for example, in blended or hybrid products mixed with conventional meat or other alternative proteins and additives, or may be grown as a stand-alone product such as ground pork, steak, or shrimp.

## 1.2 | Commercialization and stakeholder interest in food safety

The cell-cultured meat and seafood industry is growing rapidly and the pace at which technologies are moving from research and development settings toward commercialization is accelerating. By contrast, cellular agriculture as a field of research has been slower to develop, due to a lack of dedicated funding from government funding agencies, and is only beginning to be recognized as an academic research pursuit. There are an increasing number of focused research groups around the globe, some of which are receiving government support in the past year in the millions of dollars (Fell, 2020; Ministry of Business, Innovation & Employment, 2020).

The first-ever approval of a cell-cultured meat was recently granted by the Singapore Food Agency (SFA) for cell-cultured chicken used as an ingredient in a hybrid product made with plant protein (SFA, 2020). Some regulatory agencies have started to develop broad guidance for safety assessments (e.g., SFA, 2020), even though such guidance to date is not yet very detailed as the technology continues to develop. To date, regulatory agencies have relied on insight and data directly from industry, rather than from academic or third-party experts or peer-reviewed publications, partly due to the imbalance in public versus private funding in cellular agriculture today.

Continued successful commercialization requires public and private investment, efficient regulatory approval processes, and public acceptance. These milestones require assurance that products can be produced, marketed, and consumed without harm to workers, consumers, or the environment. A proactive approach employs the development of adequate evidence to assess risk and demonstrate safety in advance of commercialization. Safety testing, in particular, plays a critical role in development of safe products, regulatory authorization, and guaranteeing that statements made to investors and the public are based on sound evidence.

Safety is a key component of responsible product stewardship beyond minimum regulatory requirements, so achieving adequate safety testing is a worthy goal in and of itself. Public acceptance relies on the assumption that products are demonstrated to be safe for consumption. Regulators tasked with protecting consumers, workers, and the environment generally require safety data prior to approval. In addition, investors have an interest in ensuring that companies develop safe and compliant products so they can readily enter the market, whereas special interest groups focused on environmental issues or consumer welfare are likely to be closely monitoring developments in the cell-cultured meat and seafood market.

## 1.3 | Applicable safety concepts and methods

Safety assessment for cell-cultured products is likely to draw on general principles of risk assessment. These principles may be derived from evaluation of conventional meat and seafood products, as well as from evaluation of products in related fields, such as fermented foods, novel foods, foods produced with or from genetically engineered (GE) organisms, cloned animals, as well as drugs and medical devices. Thus, many existing safety testing methods may be adaptable to cell-cultured products. Effective risk assessment for the field is likely to require an approach that directly adopts some of these methods and modifies others, while supplementing with novel strategies.

## 1.4 | Review methodology

As cell-cultured meat and seafood is an emerging field, there are few peer-reviewed publications directly related to cell-cultured meat safety. Therefore, this review is largely based on information shared during one-on-one interviews, feedback from experts on draft documents, and discussion during a series of three virtual workshops, in addition to information synthesized from peer-reviewed articles. A series of workshops held on September 23, October 28, and December 7, 2020 were attended by 87 industry representatives from 50 cell-cultured meat and seafood companies. In parallel, interviews with industry participants and regulators were performed between August and December 2020. The initial goal of the workshops and interviews was to develop a generalized cell-cultured meat and seafood production process diagram that was applicable to most manufacturing processes. The next step was to use the diagram as a framework to identify potential chemical or biological hazards that could affect the safety of the final product and cause human health effects. Then, a review of existing principles, standards, and methodological approaches to safety testing was performed to assess relevance for cell-cultured meat and seafood products. As a result of these activities, areas requiring further research based on knowledge gaps regarding the safety of cell-cultured meat and seafood products were

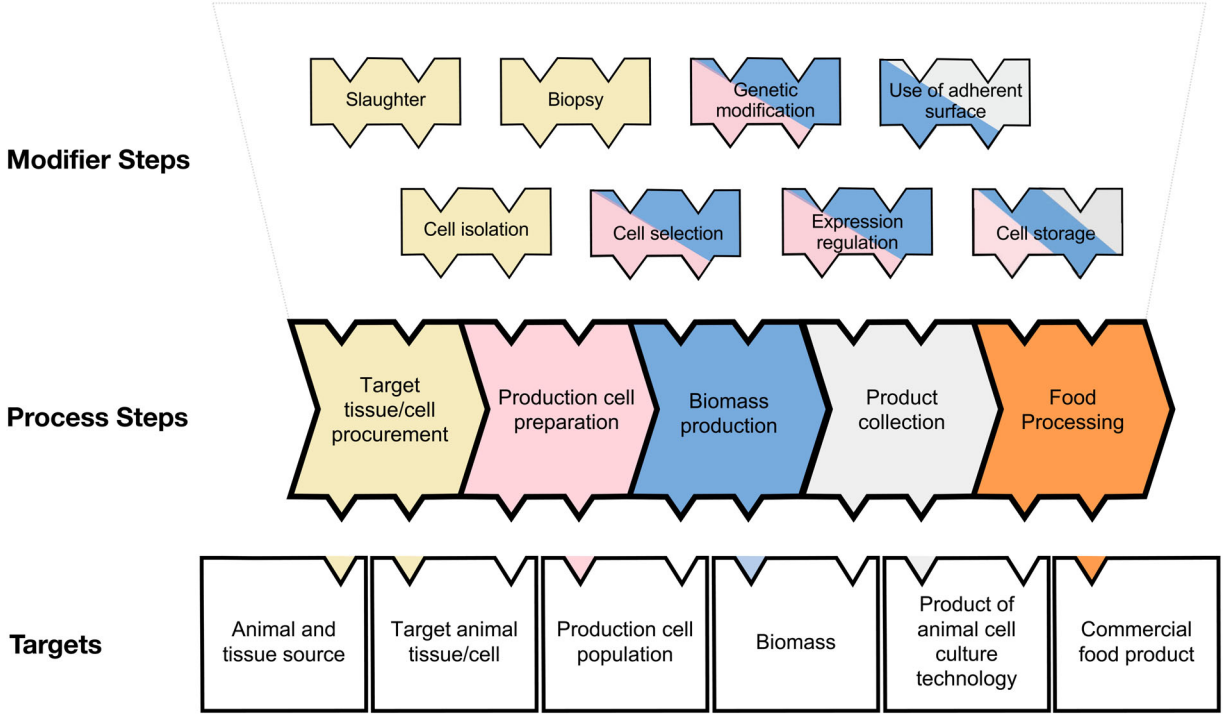


**FIGURE 1** Key steps in the cultured meat and seafood development and manufacturing process

identified. Prior to each workshop, draft documents produced by the authors of this review were circulated for comment from participants, regulators, and food safety experts for review (Supplement 1). These draft documents and feedback formed the basis of this review.

## 2 | OVERVIEW OF MANUFACTURING PROCESSES

Most cell-cultured meat and seafood technologies can be described using a generalized process diagram, shown in Figure 1. The figure was developed using a "descriptive, not prescriptive" approach, in which the diagram is designed to be modular and terminology is chosen to apply to a range of manufacturing companies across the industry. Different manufacturers may use some but not all of these steps, some may use them in unique arrangements, and others may repeat one or more steps throughout the process. The general *process steps* include *target tissue or cell procurement*, *production cell preparation*, *biomass production* (through proliferation/expansion, differentiation, or maturation), *product collection*, and *food processing*.

Further, *modifier steps* specify how the *process steps* take place with more specificity and describe different types of activities that could occur during any or none of the general *process steps*. For example, during target tissue or cell procurement, samples may be taken from live animals (*biopsy*) or from slaughtered animals (*slaughter*). *Cell isolation* may occur through isolating one cell type out of a mix of several cell types. Companies may or may not perform *genetic modification*, either for cell line enhancement or for nutritional enhancement (see Section 2.2). Various types of *expression regulation* may occur, through changing culture conditions or epigenetic or genetic changes. *Cell selection* and *cell storage* may occur at multiple steps or not at all. Some manufacturers will make *use of adherent surfaces* such as scaffolds or microcarriers. The *targets* represent the product going into and coming out of each step.

### 2.1 | Target tissue/cell procurement

Prior to the manufacturing process, tissues or cells need to be procured from live or slaughtered animals. The source cell can vary, but generally must be able to proliferate and differentiate. As a result, cell-cultured meat and seafood production relies on cells such as embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, and adult stem cells such as myosatellite cells, though some products can be produced from primary cell lines derived from specific tissues.

For tissues or cells sourced from live animals, biopsy often occurs under local anesthesia (Kadim et al., 2015; Post, 2013). Antibiotics may be used to prevent or minimize bacterial contamination (U.S. Government Accountability Office, 2020). The biopsy location will differ based on cell type and particular characteristics sought. Mesenchymal

stem cells, for instance, are commonly obtained from bone marrow or adipose tissue, whereas myosatellite cells are usually sourced from muscle (e.g., Huang et al., 2006).

Biopsied tissues may be explanted—a technique whereby the sample adheres to a plate, encouraging cell migration to the culture surface—or further processed through a combination of mechanical and enzymatic steps that liberate the cells (Post, 2013; Rubio et al., 2019). Target cells can be isolated through a range of methods, including differential adhesion, density gradient centrifugation, magnetic beads, or fluorescence-activated cell sorting (Post et al., 2020; Rubio et al., 2019).

## 2.2 | Production cell preparation

During production cell preparation, existing cells are developed into the desired starting cell types through culture and optimization.

After procurement, source cells are isolated and supplemented with culture media. Culture media generally consists of glucose, inorganic salts, water-soluble vitamins, and amino acids formulated to optimize growth for the cell type selected. Additional inputs, such as insulin, transferrin, serum proteins, growth factors, immortalization agents, antibiotics, antimycotics, and antioxidants may be used to support proliferation, differentiation, protein synthesis and degradation, and glucose uptake (Burton et al., 2000; Datar & Betti, 2010; Yao & Asayama, 2017). Some substances may be added to impart organoleptic properties (e.g., proteins or pigments that impart a meat-like color) or add nutritional value (e.g., vitamins) (Simsa et al., 2019). Media composition will influence the growth efficiency and characteristics of the final product.

Fetal bovine serum (FBS) is a common component of media, supplementing many components required for healthy cell growth (Post et al., 2020). However, due to ethical concerns related to the harvesting of FBS, along with high costs, batch-to-batch variability, and unpredictable availability, many cell-cultured meat and seafood developers are seeking alternatives to FBS. Opportunities include production of synthetic media and additives (including growth factors produced by microbial fermentation or from plant extracts) or development of bioreactor systems that mimic natural processes in the body, providing all necessary biomolecules for growth (Hanyu & Kawashima, 2016; Jochems et al., 2002; Kuo et al., 2020; Neo & Lim, 2020).

Initially, culture and optimization are likely to occur at smaller scale, in culture dishes or flasks in sterile conditions. Prior to culturing at scale, cells with especially desirable traits, natural or enhanced using genetic or expression modification techniques, are selected (*cell selection*). Similarly, a production cell population might be chosen or genetically engineered for their rapid division and resistance to senescence (e.g., Wang et al., 2019), resilience under certain environmental conditions, or other desirable features. The use of genetic modification can optimize cell nutrient efficiency, growth, and adaptation to synthetic growth media, or improve final product characteristics, such as nutrition, taste, and texture (Ben-Arye et al., 2020; Lee et al., 2016; Rubio et al., 2019; Stout et al., 2020).

Rapid and prolonged proliferation is crucial to developing cell-cultured meat and seafoods, so replicative ability is likely to be prioritized during cell selection. The so-called Hayflick limit—the finite number of cell divisions possible before death—can be extended or overcome by regularly replenishing the cells in the culture, optimizing cell media with factors capable of extending proliferative capacity, increasing regenerative potential, or fully immortalizing the cell line (Datar & Betti, 2010; Edelman et al., 2005). These latter strategies might be achieved via genetic engineering, through selection of cells that express immortality (achieved via spontaneous mutations), or the addition of various immortalization reagents such as those that activate the telomere lengthening gene hTERT, upregulate cell-proliferation genes Myc and Ras, or inactivate tumor suppressor genes such as p53 and Rb (Gudjonsson et al., 2004; Ramboer et al., 2014; Sears et al., 2000; Stephens et al., 2018; Yang et al., 2007).

## 2.3 | Biomass production

Manufacture at scale begins with biomass production, which may include proliferation, differentiation, and/or maturation of cells to increase the biomass.

During the proliferation step, cells are placed in bioreactors of increasing size to grow and replicate for commercial production. There are currently no specific bioreactors considered the industry standard; multiple bioreactors may be used for different steps of a process, each optimized for specific steps.

Bioreactors are closed, automated systems that contain culture media and allow for precise control over biologically relevant variables, including physical (e.g., temperature), chemical (e.g., oxygen concentration), and biological (e.g., cell density) conditions. As the cells and tissues grow, nutrient and energetic needs change, thus the cell media composition may change throughout the process (Bellani et al., 2020; Post et al., 2020). Adequate oxygenation may be particularly challenging at scale, necessitating the use of dedicated oxygenation systems within bioreactors along with the addition of oxygen carriers such as modified hemoglobin/myoglobin or perfluorochemicals (Datar & Betti, 2010; Simsa et al., 2019).

Following proliferation, cell differentiation is triggered to obtain the desired mature cell type. This may be achieved by changing the scaffold, the culture media, and/or the environmental conditions (Datar & Betti, 2010; King & Miller, 2007; Levenberg, 2019; Stephens et al., 2018). Various vitamins, growth factors (such as TGF-$\beta$1, FGF2, IGF-1, etc.), proteins, amino acids, trace minerals, and other small molecules may be added or removed from the culture media to direct differentiation and expression of various molecules (Braga et al., 2017; Datar & Betti, 2010; Stern-Straeter et al., 2014).

Most cells used in cell-cultured meat and seafood processes are anchorage dependent, meaning that they require an adherent surface on which to attach and grow. To provide this structure, some processes may rely on microcarriers—small beads (generally in the range of 100–300 μm) composed of materials such as polystyrene, gelatin, dextran, or collagen that provide an anchorage surface for cells suspended in culture media (Chen, Reuveny, et al., 2013; McKee & Chaudhry, 2017). Structured food products may require more robust scaffolds.

Scaffolds may be produced through a range of methods, including recombinant technology, fermentation production methods, extrusion, and bioprinting (Ben-Arye et al., 2020; Courtenay et al., 2018; Widhe, 2016). Scaffold materials can be natural, synthetic, or composite and are ideally biocompatible, edible, safe, and provide suitable texture to the final product. Scaffold materials might include natural biomaterials such as polysaccharides such as cellulose, decellularized plants, alginate, and chitosan; proteins such as collagen and gelatin (which may or may not be sourced from animals); and textured soy protein (Ben-Arye et al., 2020; Bodiou et al., 2020; MacQueen et al., 2019). Synthetic materials are usually composed of polymers, such as polyethylene glycol or polyacrylamide. Adherent surface materials may be functionalized to promote adhesion and create favorable surface characteristics; scaffolds may be altered to enable co-cultures of different cell types or to integrate or mimic cell adhesion motifs. This may be achieved by including biomaterials, chemically crosslinked peptides, or GE biomaterial scaffolds (Campuzano & Pelling, 2019; Davidenko et al., 2015).

## 2.4 | Product collection

After the desired biomass is grown, the product is collected. In the medical and pharmaceutical industries, harvesting requires maintaining cell viability; this is not important for food. For products that require removal from scaffolds or microcarriers, cells may be removed enzymatically, chemically, and/or mechanically (Bodiou et al., 2020; Nienow, 2014). Dissociation agents (e.g., trypsin–EDTA) may be used for enzymatic or chemical removal; mechanical approaches may include agitation, flocculation, or sedimentation. Some carriers can be separated from the cells through chemical degradation (Bodiou et al., 2020). There is a current need for automated harvesting systems that reduce the need for manual harvest (Specht et al., 2018). Some products may include edible scaffolds such as textured soy protein that are embedded within the final product (Ben-Arye et al., 2020). Ideally, after cells are harvested, media is removed from the bioreactors, reformulated, and recycled for future use (Datar & Betti, 2010).

## 2.5 | Food processing

After the cells or tissue products are harvested, the animal cell-cultured product is formulated into commercial food products through food processing. Products will likely mimic existing products, such as hamburgers, meatballs, nuggets, or whole meats such as steaks, chicken wings, shrimp, or fillets. Some products will not be made entirely of cell-cultured products—cultured cells may be mixed with binders, flavors, conventional food additives and ingredients, fermentation-produced additives, plant-based products, or other cell-cultured products (U.S. Government Accountability Office, 2020). The exact nature of processing will vary based on the desired product, but may include processing methods used for conventional foods, such as sterilization, heat or radiation treatment, fermentation, enzyme treatment, pickling, smoking, drying, curing, pasteurizing, high-pressure processing, and modified atmosphere packaging (European Food Information Council, 2016). New meat processing techniques may be developed, or techniques common to the meat and seafood industry repurposed; for example, using enzymes such as transglutaminase to combine different tissues (Kieliszek & Misiewicz, 2014).

## 2.6 | Cell storage

To ensure cell line stability and a consistent product, most processes will likely rely on cell storage in stock cell banks to avoid repeated procurement from animals and to ensure consistency in the cell type. Cell banks may be created from isolated primary cells or cells further in the development process; banking may occur multiple times throughout the process.

Cells are selected, validated, and frozen in small batches that can later be thawed, validated again, and expanded to produce the desired product. The preservation process may rely on vitrification—rapid freezing that reduces the risk of intracellular crystallization—or on other methods,

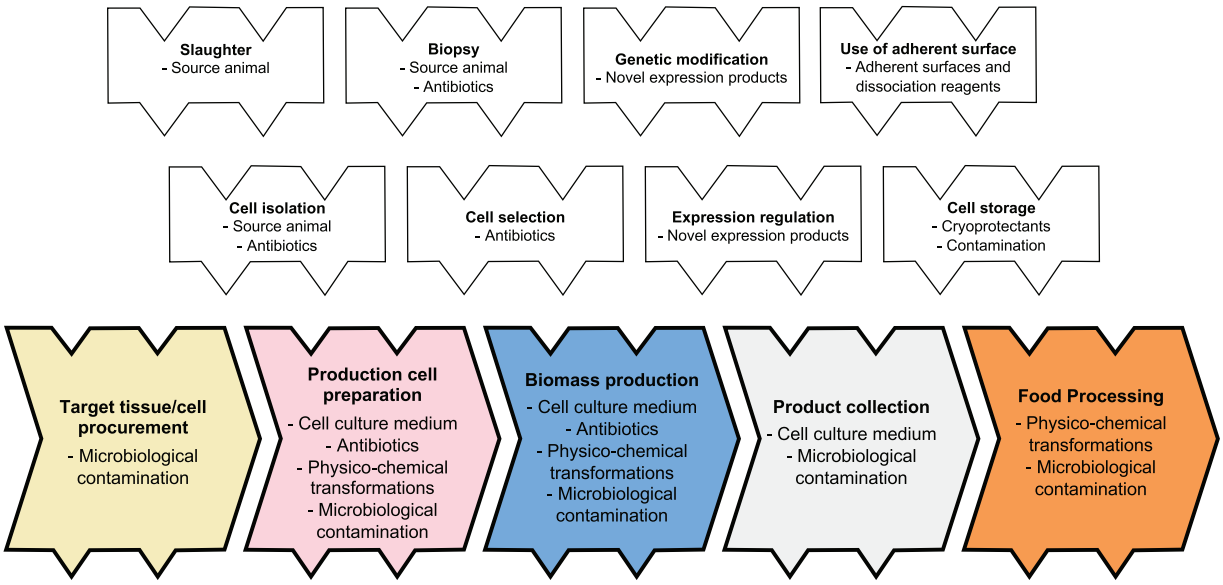


**FIGURE 2** Key hazard considerations related to different process steps during manufacturing

many of which have been developed for clinical applications (Andrews et al., 2015; Hunt, 2011). Cell products are stored at low temperature (often under liquid nitrogen), and cryoprotectants are used to protect the cells (Elliott et al., 2017).

## 3 | MANUFACTURING PROCESS HAZARD CONSIDERATIONS

Cell-cultured meat and seafood production may rely on manufacturing methods not currently used for food, which means the inputs, byproducts, and outputs from each manufacturing step require assessment to identify potential safety concerns. In this section, potential hazards are identified for each process step; some hazards may be present throughout the process, whereas others are specific to only one step. Figure 2 links the manufacturing process hazard considerations to specific manufacturing steps, and Table 1 summarizes sources and the identified hazards, as further detailed in this section.

### 3.1 | Microbiological contamination

Microbiological contamination with bacteria (including mycoplasma), fungi, and viruses can occur throughout the entire manufacturing process. Mycoplasma contamination may be of particular concern. Mycoplasma are small bacteria that can infect a wide range of hosts and be pathogenic. They are common cell culture contaminants, estimated to infect 5%–35% of the world's cell lines (Nikfarjam & Farzaneh, 2012). They are resistant to antibiotics, can pass through filters, and grow slowly so may not be detected for months or even years in continuous cell cultures (Drexler & Uphoff, 2002). Source animals, personnel, other cell cultures, cell media components, equipment and supplies, and liquid nitrogen can all be sources of mycoplasma contamination and spread (Nikfarjam & Farzaneh, 2012).

Meat manufactured through conventional practices is prone to contamination from bacteria such as *Salmonella*, *Listeria*, and *Escherichia coli* that reside on animals, in the digestive tract, and in feces (Rhoades et al., 2009). The prevalence of these microbes generally decreases as the carcasses are washed and disinfected and meat is processed, and contamination can be prevented with careful evisceration (Rhoades et al., 2009). Regardless, a quantitative assessment of bacterial contamination of beef carcasses and retail meat by Martínez-Chávez et al. (2015) revealed *Salmonella* on 18% of beef carcasses and 71% of ground beef samples, and *E. coli* on 97% of beef carcasses and 100% of ground beef samples. *Salmonella* counts were in the order of 2 log CFU/25 g beef, and *E. coli* in the order of 4 log CFU/25 g ground beef. A 4-log concentration of *E. coli* in the equivalent amount of animal cell culture would inevitably outgrow that cell culture rapidly and result in disposal of that batch. In theory, cell-cultured meat and seafood products are less susceptible to contamination arising from slaughter, presenting reduced risks of microbial foodborne disease and resistance to deterioration and spoilage once manufactured. However, contamination can occur throughout the cell culture manufacturing process and needs to be controlled.

Raw materials and added reagents may include bacteria, fungi, and viruses that can contaminate cells. Therefore,

**TABLE 1** Summary of source, main potential hazards, and potential outcomes that may require investigation

| Source | Main potential hazard(s) | Potential outcomes |
|---|---|---|
| Source animal | Bacteria, viruses, parasites, prionsAntibiotics | Introduction of infectious disease agents into cell culture.Increase in antibiotic resistance. |
| Cell culture medium | Fetal Bovine Growth serum and animal-derived components<br>Antibiotics<br>Inputs at higher concentrations than found in conventional meat or seafoodNovel inputs and allergens | Introduction of infectious disease agents into cell culture from cell culture components.<br>Increase in antibiotic resistance.<br>Could be hazardous to human health (e.g., certain growth factors).<br>Could be hazardous to human health.Potential allergenicity. |
| Cell storage inputs | Cryoprotectants | Final product contains cryoprotectants in amounts not safe for human consumption. |
| Cell storage conditions | Leakage of cryopreservation fluid into cells | Microbiological contamination or cross-contamination of cells. |
| Continual subculturing, handling, and transferring of cells | Microbiological contaminationPhysicochemical transformations | Introduction of infectious disease agents into cell culture.<br>Changes in cell morphology, function, and physiology may result in a final product that has characteristics different to those of conventional meat. |
| Novel expression products | Hazardous or allergenic proteins or bioactive moleculesIntroduction of traits of concern | Alterations in the types and levels of endogenous gene expression or as a result of genetic drift may cause pleiotropic effects or novel expression products that may not be safe for consumption.<br>May result in traits of concern, such as antibiotic resistance. |
| Scaffold and microcarriers | Hazardous materials | Materials used for adherent surfaces and their degradation products may not be safe for consumption. |
| Dissociation reagents | Hazardous reagents | Use of hazardous reagents may end up in the final products. |
| Food processing | Physicochemical transformationsNovel inputs and allergens | Induction of structural and chemical changes different from those of conventional meat.<br>Could be hazardous to human health.Potential allergenicity. |
| Equipment, supplies, packaging, cleaning products | Chemicals<br>Microbiological contaminationAllergens | Leaching of hazardous chemicals or substances into cell culture.<br>Introduction of infectious disease agents into cell culture.<br>Cross-contamination with allergenic substances. |

controlling for the identity, purity, and (when possible) the sterility of the inputs and evaluation of their safety will be essential to ensure safety of the final product.

Caution is needed to avoid contamination during handling, preparation, and transferring of cells, contact with contaminated equipment, or during water bath thawing throughout the cell storage process (Cobo et al., 2005; Fountain et al., 1997; Thirumala et al., 2009). Fully enclosed equipment is desirable from a safety perspective, as it allows for improved control and monitoring of potential contaminants. As such, bioreactors are anticipated to create ideal, closed environments that are not prone to contamination, but careful design may be needed to realize these advantages. Cells need to be handled aseptically and continually monitored for microbial growth and contamination.

Leakage of cryopreservation bags in liquid nitrogen during cell banking can lead to cross-contamination (Tedder et al., 1995), and liquid nitrogen itself has the potential to transfer pathogens to cells, even if stored in freezing bags (Fountain et al., 1997). Storage of cell banks in the vapor phase (as opposed to the liquid phase) of liquid nitrogen may limit the potential for cross-contamination (U.S. Food and Drug Administration, 2010).

In final food processing steps, microbiological contamination may be introduced from the processing methods themselves, from addition of other ingredients, or from the packaging process. Assessment may be needed regarding whether food processing treatments such as heat treatment, high-pressure processing, and irradiation are needed to adequately control potential microbial contaminants.

## 3.2 | Source animal

The relationship between the health status of the source animal and the potential to introduce biological hazards into cell-cultured products is yet to be researched. Possible issues include transmission of infectious diseases and prion propagation. Infectious viral disease can be transmitted as free viral particles through fecal contamination of food (e.g., norovirus, avian influenza) or via transmission of infected cells to other hosts (e.g., hepatitis A, hepatitis E, bovine leukemia virus) (Buehring et al., 2019; de Graaf et al., 2016; Espinosa et al., 2020; Olaya-Galán et al., 2017). Research suggests that viruses may be able to propagate or at least persist under some conditions (Gillet et al., 2004; Graves & Ferrer, 1976). Further, if cells from an animal infected with a retrovirus are biopsied and cultured, it is unknown whether the retroviral DNA of the infected cells will persist in culture. Parasites are associated with some food animals and if consumed, some can cause illness or disease. Some of the more common foodborne parasites include *Taenia solium* from pig and wild boar; *Toxoplasma gondii* from small ruminants, pig, cow, and game; *Trichinella spp*. from pig and game; *Opisthorchiidae* from freshwater fish; and *Paragonimus* from freshwater crustaceans (FAO/WHO, 2014). Inspection of source animals and biopsied tissues for signs of infection will likely limit the chance of contamination. The potential for zoonotic viruses (i.e., viruses capable of animal-to-human transmission) and parasites and their ability to persist or proliferate during cell-cultured meat and seafood manufacture warrants further research.

Prions are infectious agents responsible for some neurodegenerative diseases in humans (e.g., Creutzfeldt–Jakob disease [CJD]) and animals (e.g., scrapie in sheep and goats, bovine spongiform encephalopathy [BSE] in cows, chronic wasting disease in deer and elk) (Gough & Maddison, 2010). It may be possible to contract variant CJD from eating the meat of diseased cattle (Chen, Wang, et al., 2013). Prions have been found in the brain, spinal cord, lymphoid tissues, tonsil, appendix, enteric nervous system, and the blood of afflicted animals (Gough & Maddison, 2010). If these are not used as source tissues for cell-cultured meat and seafood, and if the source animals are certified BSE free, the risk of capturing and propagating prions is low. There is currently a lack of understanding of the exact mechanisms of prion function, tissue distribution, and transmission paths; as such, improved understanding of detection of prions in animals and the hazards associated with using prion-infected tissue for cell-cultured meat and seafood will further improve stakeholders' ability to control risk.

## 3.3 | Cell culture medium

Cell-cultured meat and seafood require cell culture media from target tissue/cell procurement through to product collection. Characterizing the components of the media to identify potential hazards is therefore an important part of safety assessment. This endeavor is complicated by the different types of media required for different species, cell types, and for different stages of manufacture (Burton et al., 2000; Yao & Asayama, 2017). There are hazards associated with the intentional components and from impurities or contaminants.

Animal-origin cell culture reagents, such as trypsin, gelatin, collagen, and amino acids, may introduce viral or prion contamination (Jayme & Smith, 2000; Marcus-Sekura et al., 2011). Commercial sera such as FBS have been found to harbor various contaminants, such as viruses, prions, bacteria, yeast, fungi, and endotoxins stemming from infected animals from which the FBS was sourced, or contamination during laboratory manipulation (Cobo et al., 2005; Erickson et al., 1991; Tekkatte et al., 2011). Studies have found that between 20% and 50% of commercially available FBS contain viruses (Wessman & Levings, 1999). For example, bovine viral diarrhea virus is regularly detected in FBS and can be propagated during vaccine or biotherapeutic production (Laassri et al., 2018). Although this virus is not known to cause disease in humans, it is indicative of a need to properly characterize the source of raw materials to avoid potential contaminants. Some viruses may be zoonotic disease agents and may be able to replicate in vitro (Marcus-Sekura et al., 2011), warranting further research. FBS is a complex mixture of thousands of components (Post et al., 2020), and most formulations are not well characterized (or the characterization is not publicly available). The exact composition of commercial media can vary batch-to-batch (Gstraunthaler & Lindl, 2013; van der Valk et al., 2010). In part because of these challenges (along with other issues such as high cost, limited availability, and animal welfare concerns), synthetic or serum-free media are being extensively studied for commercial use in cell-cultured meat and seafood. Media components are anticipated to be similar to those in current use (e.g., Essential 8™ medium) and are expected to use existing food ingredients, such as vitamins, growth factors (such as TGF-$\beta$1, FGF2, IGF-1, etc.), proteins, amino acids, hormones, trace minerals, and other small molecules (Kolkmann et al., 2020; Specht et al., 2018). Media constituents typically derived from animals may be substituted with nonanimal components to limit introduction of pathogens (Jayme & Smith, 2000). In theory, serum-free culture media would allow for optimization and control over components, resulting in an

improved ability to test cell media safety (McGillicuddy et al., 2018).

Any component of the cell culture medium has the potential to become part of the final food product. Byproducts or residues can be left from the substance itself or any other substances formed in or on food as a result of the compound's use during manufacturing. The use of novel ingredients or existing components at higher concentrations than in conventional meat or seafood may raise additional safety concerns. For example, the use of wheat gluten as a hydrolysate (Radošević et al., 2016) may result in allergens in the final product. Cell-cultured meat and seafood product manufacture likely requires addition of signaling molecules such as growth factors (Ben-Arye et al., 2020; Tekkatte et al., 2011). These substances may also be naturally produced during the culturing process, and their production may also be stimulated through expression regulation or genetic engineering. Although hormones fulfill an essential role in normal body function, consuming excess or additional hormones from food may cause imbalances and result in adverse human health outcomes, such as reproductive and developmental toxicity or pro-carcinogenic effects (Jeong et al., 2010; Scientific Committee on Veterinary Measures relating to Public Health, 2002). High concentrations of some growth factors, such as IGF-1, have been linked to increased risk of certain cancers (Renehan et al., 2004; Vasconcelos et al., 2019). In conventional farming, the use of growth hormones is restricted in some markets. For example, the European Union bans some substances that have hormonal or thyrostatic actions, as well as ß-agonists (European Council, 1996); these substances can pose human health concerns because they may remain as residues in meat. The levels of signaling molecules used in cell-cultured meat and seafood may be lower than that of traditional livestock farming. Further, due to interspecies differences, it is uncertain if there will be any biologically meaningful interaction between the substances used to manufacture cell-cultured meat and seafood products and human receptors (consumers). Further, growth factors may be destroyed during digestion or food processing stages, especially with heat treatment. Some growth factors may be resistant to such degradation, as demonstrated in pasteurization of breast milk (Escuder-Vieco et al., 2018; Ewaschuk et al., 2011); however, there is little research on meat and seafood.

Accumulation of certain cell media components may occur due to molecules being added to cell cultures or secretion from the cells (e.g., growth factors). Some manufacturers may recycle their cell culture media, resulting in further accumulation. Due to ongoing optimization of the manufacturing process, identifying which molecules may be of human health concern, determining the residue concentrations in the final product, and comparison to levels in conventional foods is important to confirm that no human health effects would be anticipated.

## 3.4 | Antibiotics

The initial cell isolation procedure may require tissue or cell collection from live or recently slaughtered animals in nonsterile environments. This activity is susceptible to microbial contamination, and antibiotics may be necessary to control bacterial, fungal, or yeast growth (Cobo et al., 2005). Antibiotics are also sometimes added to cell culture media to reduce contamination risk. As in conventional agriculture, antibiotics used in cell-cultured meat and seafood may persist in the final food product.

In conventional livestock farming, antibiotic use has been criticized for accelerating antibiotic resistance in microorganisms; additional concerns include the release of excess or runoff antibiotics into the environment, as well as consumer exposure to residual antibiotics that remain in the final product, which is less studied (Chen et al., 2019; Jeong et al., 2010). On a societal scale, antimicrobial resistance could lead to an inability to manage outbreaks and treat diseases with antibiotics (Centers for Disease Control & Prevention, 1994; National Research Council, 1999; Okocha et al., 2018). In terms of individual risk to human health, unintended consumption of residual antibiotics may lead to drug resistance or hypersensitivity, or other direct effects such as allergic reaction, harm to gastrointestinal flora, or carcinogenic, mutagenic, and teratogenic effects (National Research Council, 1999; Okocha et al., 2018).

Many producers are avoiding antibiotic use altogether by working in sterile and carefully controlled conditions (e.g., FSIS/FDA, 2018). However, if used, antibiotics will be at a lower concentration than in conventional animal farming, and used at earlier stages of manufacturing, where the cells will then be rinsed and purified, reducing the chances that antibiotics will persist in the final product. They are generally consumed at lower concentrations in food than when consumed directly and rarely result in allergic reactions (National Research Council, 1999). It is important to document use, and characterize the types and concentrations of antibiotic in the final product that result from cell-cultured meat and seafood manufacturing processes to assess whether adequate human health safety data are available and to limit any contribution to antibiotic resistance.

## 3.5 | Cryoprotectants

Some cryoprotectants used during cell storage may be toxic if they end up in the final food. Cryoprotectants such as

inulin, sorbitol, and dimethyl sulfoxide are already used as food processing aids and demonstrated to be safe at certain concentrations in food (MacDonald & Lanier, 1997; Savini et al., 2010), though it is conceivable that novel cryoprotectants will be used. For cell-cultured meat and seafood purposes, it is expected that cryoprotectants will be washed away or diluted to very low concentration by the time the final product has been developed. Regardless, due to the potential toxic effects, the cryoprotectant used in the freezing process will likely need to be evaluated for safe use.

### 3.6 | Physicochemical transformations

Continual subculturing of cell lines may result in changes in morphology, function, and proliferation rate due to physiological adaptation or genetic drift, thereby increasing the chance that the passaged cells have different characteristics from the original primary cell type (Hughes et al., 2007; Specht et al., 2018). It is conceivable that in the future, cells are purposely developed to have unique physicochemical properties not seen in conventional meats. As such, the cells themselves require physicochemical inspection throughout the process and ultimately be tested for safe consumption.

It is unknown whether some cell-cultured meat and seafood products will respond to food processing differently than conventional products. Structural and chemical changes such as oxidation, resistance to degradation, enzymatic activity, alterations in microstructure, and so forth may differ, especially in products with novel components, such as synthetic scaffolds. Further, conventional meats have microbial populations that will be different than those associated with cell-cultured products. Organoleptic characteristics, product stability, and spoilage can be influenced by this microbial community (Doulgeraki et al., 2012; Frank et al., 2020; Yang et al., 2020). It is unknown how the differences in cell-cultured meat and seafood microbiota may affect physicochemical properties of cell-cultured meats, affecting aspects such as shelf life, storage conditions, and safety of the final product.

### 3.7 | Genetic modification and novel expression products

Genetic modification can be achieved by traditional techniques, such as mutagenesis and/or selective breeding, or genetic engineering (e.g., via genome editing). In addition, completely unintentional genetic alteration may occur, for example, through genetic drift after multiple passages of cells, a phenomenon that may raise concern unless monitored and controlled. The effects of genetic drift may be minimized by using fresh vials of banked cells for each batch, which is common practice in fermentation with microbial production strains (Sewalt et al., 2016). The nature and types of genetic alterations require further research to evaluate which types of changes may pose food consumption hazards, and to what extent.

Some cell-cultured meat and seafood technologies rely on genetic engineering techniques leading cells to exhibit new or altered expression of traits (WHO, 2008). Where they do, the safety of the GE cell line and its products will need to be evaluated. For example, cell line immortalization (or at least cell life extension) can be achieved by biotechnological methods involving oncogenes, but concerns remain regarding genomic stability and potential tumorigenicity after genetic modification (Wang et al., 2019), which would be useful areas of further research.

Alterations in expression levels or the expression of novel products can be triggered through changes in cell media or culture conditions, through genetic modification, and/or through epigenetic modifications. Some products may be specifically engineered to produce or alter current levels of certain nutrients or remove antinutritional properties. These alterations may be employed to reduce adverse human responses; for example, for those with the meat allergy alpha-gal syndrome, removal of alpha-gal sugars on the surface of cells reduces the chance of allergic reaction to meat products (U.S. Food and Drug Administration, 2020). The novel expression or upregulation of proteins or bioactive metabolites not normally synthesized in animals may improve nutritional value and food quality, such as antioxidant carotenoids (Stout et al., 2020). However, an assessment of whether these expression products are safe for human consumption is needed.

GE microorganisms have been used for decades for fermentation of a range of common food enzymes and food ingredients, including proteins, enzymes, vitamins, amino acids, organic acids, and flavors (Adrio & Demain, 2010; Hanlon & Sewalt, 2021; Sewalt et al., 2016). One difference between animal cell-cultured meat/seafood production and microbial ingredient production is that microbial cells and their genetic material are commonly removed from the final product or are present at low levels, whereas GE cells could form the bulk of the final cell-cultured food product. Environmental safety concerns regarding the potential to release GE organisms (e.g., during disposal) and allow intermixing with wild species have been raised (Van Slyck, 2017). In the case of cell-cultured meat and seafood, the potential for environmental spread of modified genetic material may need to be evaluated, though significant effects would not be expected. The cells generally require controlled and specialized environments to survive and are not anticipated to multiply in natural environments. Products of cell-cultured meat and

seafood are no longer maintained in a living state once they reach consumers, and therefore may be less likely to actively transfer modified genetic material to natural populations. There is limited evidence that suggests that DNA from dietary sources can integrate or be transferred into somatic cells or the microflora of the human gastrointestinal tract (Nawaz et al., 2019). However, research has largely focused on transgenes from consumed GE plants, with less work focused on transfer from consumed animal cells, which represents a gap in knowledge in this field. In particular, evaluation of the extent to which modified genetic material of concern can be transferred to gut microorganisms, and studies with focus on animal cells relevant to cell-cultured meat and seafood, is needed.

### 3.8 | Adherent surfaces and dissociation reagents

Scaffold, microcarriers, and chemical and enzymatic dissociation reagents for removal of cells from the scaffold need to be assessed for safety. Generally, these additives are anticipated to meet food-grade specifications because they may end up as part of the final product (Stephens et al., 2018). The synthesis of scaffolds sometimes involves chemical crosslinking agents such as formaldehyde and glutaraldehyde, enzymes, or photoinitiators to make them suitable for tissue engineering (Oryan et al., 2018). Human tissues can be particularly sensitive to these chemicals, even at low concentrations (Fürst & Banerjee, 2005). The use of safer crosslinking agents is desirable, or alternatively flushing the toxic components and testing for presence in the final product is required.

If the scaffold is designed to degrade, transform, or interact in such a way that produces unsafe components, then these byproducts or degradation products require safety assessment. If materials have not been demonstrated to be safe for use in food then, they will likely require a full safety assessment typical for any food additive or ingredient.

### 3.9 | Other chemical substances

Inorganic and organic leachable substances and chemicals from disposable products (e.g., cell culture plastics, filters), coatings on equipment, packaging materials, and cleaning products have the potential to migrate and leave residues in food. At minimum, using food-grade materials and maintaining documentation of the supply chain can inform the testing required in the final product.

## 4 | METHODS TO MANAGE AND ASSESS SAFETY

### 4.1 | Manufacturing process safety

Cell-cultured meat and seafood safety depends on a manufacturing process designed with product safety in mind and an assessment of the final product. This section describes practices and protocols from related fields that can be translated to the cell-cultured manufacturing context as part of creating safe, consistent, high-quality products.

#### 4.1.1 | Good Manufacturing Practice

Good Manufacturing Practice (GMP) relates to the overall "good housekeeping" principles intended to prevent a hazard from occurring and is a set of widely applied food production practices that describe appropriate design and construction of facilities, sanitary operations and maintenance, and production and process controls that ensure reliable results and safe production of food (21 C.F.R. §, 117, 2015; Blanchfield, 2005; De Oliviera et al., 2016). GMPs and standard operating protocols from the food, feed, meat and seafood, and pharmaceutical and medical fields (e.g., De Oliviera et al., 2016) can be applied to cell-cultured meat and seafood manufacturing to ensure consistent quality and safety of the product. In addition, Good Hygiene Practices (GHPs) are essential in the food supply and can be audited alongside GMP compliance. GHPs extend beyond industrial food manufacture into the service industry, such as catering, hotels, and restaurants

#### 4.1.2 | Good cell culture practice

Also applicable to cell-cultured meats and seafoods are concepts from Good Cell Culture Practice (GCCP). These principles are typically applied to in vitro systems for basic research, medicines, and pharmacology to maximize the reliability of cell and tissue products but some aspects are relevant for handling and management of cell-cultured meat and seafood (Bal-Price & Coecke, 2011; Hartung et al., 2002). GCCP sets minimum standards and provides recommendations for in vitro work to prevent contamination and ensure quality of the final product. Among other relevant recommendations, GCCP suggests working with aseptic techniques and avoiding antibiotic use, developing standard operating procedures, and controlling the quality of media supplements and other inputs. Documentation is emphasized; maintaining a detailed record of all procedures can provide information on what potential contaminants or hazardous inputs may be present in the final

product, which can support targeted screening for potentially harmful impurities and contaminants. The standards set by GCCP is likely prohibitive for food; however, development of "food-grade" GCCP based on existing guidelines may be a good next step.

#### 4.1.3 | Code of hygienic practice

Until the relationships between source animal health and final food product are understood for cell-cultured products, guidance regarding the health of food animals or recommendations related to the use of animal-derived materials for medical procedures may be useful. A code of hygienic practice already exists for animal food production, which includes procedures for herd management to maintain animal health and prevention of animal disease (WHO, 2008). Animals used as source animals for xenotransplantation (i.e., use of live cells, tissues, or organs from an animal source in a human recipient) are recommended to be healthy and reside in specific pathogen-free closed herds with health screening programs (European Commission, 2006; U.S. Food and Drug Administration, 2010, 2016). These programs track and monitor infectious diseases, and documentation of animal health history is required. This proactive approach can be especially useful where there are no validated screening tests to detect endogenous pathogens. This is the case, for example, for prion-associated diseases, such as BSE where diagnosis can only be made in a postmortem examination of brain tissue (U.S. Food and Drug Administration, 2019). Active herd/flock management and documentation, along with monitoring and screening of source animals for potential infectious disease, will lower the risk of culturing affected cells. Similarly, isolation of animal-derived components of cell media (e.g., BSA, trypsin, collagen, etc.) from low-risk animals reduces the chance of contamination (Jayme & Smith, 2000).

#### 4.1.4 | Hazard and risk management systems

Management systems can help prevent or minimize hazards and manage specific risks within a process. This review identifies some potential biological and chemical hazards; as more data are developed and manufacturing processes evolve, other hazards (e.g., physical) and associated risks (i.e., the likelihood that a hazard poses a significant safety issue) can be assessed. Systems to manage food safety risk such as Hazard Analysis and Critical Control Point (HACCP), Hazard Analysis and Risk-Based Preventative Controls (HARPC), Food Safety Plan development, and other risk-based preventative controls programs may be applied to cell-cultured meat and seafood processes. In each approach, a systematic review is performed for each step of the manufacturing process to identify every possible hazard or source of contamination. A control or procedure is introduced to prevent, eliminate, or minimize each hazard based on its risk (e.g., Johnston, 2000). In many countries, a risk prevention system is a regulatory standard or law and is often considered essential to achieving greater market access (e.g., European Commission, 2004a; U.S. Food and Drug Administration, 1997). Detailed documentation of each process step and identification of potential hazards can help identify the impurities and contaminants that warrant examination in the final product. This review delivers a first step toward hazard identification, recognizing that it is premature to conduct a generalized risk assessment.

#### 4.1.5 | Input materials and equipment selection

Input material selection and control provides a second example where more general frameworks can be supplemented with practices from specific fields. Cell culture materials can be selected to comply with current GMP and food-grade specifications. The selection and management of equipment, disposables, and cleaning agents made of food-safe materials will limit the amount of toxic extractables and leachables migrating into the product. Standardized tests for such contaminants to ensure quality and safety can be drawn from the biopharmaceutical, medical device, and cosmetics industries, where much has already been established related to testing regimes for process-related contaminants and residue measurement (Gao & Allison, 2016; International Life Sciences Institute, 2015).

#### 4.1.6 | Contaminant control

Every new cell line can be cultivated in a quarantine incubator and verified that they are pathogen free. Microbiological controls and testing derived from practices involving stem cells or in vitro practices can be applied throughout the manufacturing process. Many methods exist to evaluate and reduce contamination from infectious agents introduced via equipment, handling, material inputs, or processes where cultures are exposed to the air. A system of daily observation and regular screening of cultures, media, and equipment using standard protocols can be adapted from those provided in regulatory guidance or pharmacopeial standards (Cobo et al., 2005). Investment in rapid microbiological testing and implementation of effective controls and procedures to limit

contamination is essential. Viruses and other undesirable agents can be reduced or removed from serum and final products through heat inactivation, irradiation, or filtration (Jayme & Smith, 2000; Laassri et al., 2018). In addition, cells intended for banking may be screened for bacteria, yeast, fungi, prions, and viruses to prevent unintentional propagation in future batches (Cobo et al., 2005; European Commission, 2004b; U.S. Food and Drug Administration, 1998; U.S. Food and Drug Administration, 2010).

## 4.2 | Demonstrating safety of cell-cultured meat and seafood

The extent of toxicity testing required for cell-cultured products or specific inputs in the manufacturing process is yet to be established. To establish the safety of the final product (which includes cell-cultured meat and seafood as an ingredient, additive, or a whole food), a safety assessment of the inputs and then an evaluation of the types and levels of residues, byproducts, and metabolites remaining in the final product will be necessary. If deemed to have significant novel or unique properties, an assessment of the final product itself as a whole may be needed.

Many existing standard toxicity testing methods may be used to assess inputs. Generally, any inputs into food must be of food-grade quality, meeting specifications and criteria specific to that ingredient (e.g., as specified in the Codex Alimentarius). Development of specifications for cell-cultured meat and seafood additives, such as scaffold materials, may be warranted.

Approaches to safety testing of ingredients and food additives are well established, using biochemical, in silico, in vitro, and in vivo methods, as described in the next sections. Globally harmonized testing standards—such as those developed by the Organisation of Economic Co-operation and Development (OECD), World Health Organization (WHO), Food and Agriculture Organization (FAO), or regulatory organizations—may be applied directly or modified for use in the cell-cultured meat and seafood safety testing context. Tests and analyses under these standards are generally carried out following Good Laboratory Practice (GLP), a set of principles designed to assure study quality and integrity.

Products that have compositional, nutritional, and functional equivalency to already accepted foods are in theory as safe as the products to which they are equivalent (European Food Safety Authority, 2008; U.S. Food and Drug Administration, 2008). The comparison then relies on the history of safe use and data supporting the safety of the conventional food. Any identified differences will direct further safety testing (Constable et al., 2007; European Commission, 1997; FAO/WHO, 2000). It is expected that some cell-cultured products will not be exactly the same as their conventional counterparts. For example, cell-cultured products may contain synthetic scaffold materials or other novel inputs, the cells may be genetically modified such that new proteins are expressed or existing proteins are under- or overexpressed, and the biochemistry and composition of those proteins may vary. Accordingly, toxicity testing may be required to demonstrate the safety of inputs and components in the final product.

### 4.2.1 | Microbiological analysis

Typically, microbiological limits are established for conventional livestock or aquaculture products (e.g., Government of Canada, 2020). Guidance has been developed to help identify microbiological hazards in meat, poultry, seafood, and other animal proteins (U.S. Department of Agriculture, 1999). Bacterial and viral contamination may be detected through routine process monitoring; physicochemical changes, pH shifts, changes in turbidity, and compromised cell cultures can signal contamination. Existing standards, guidelines, and specifications for microbiological characterization are likely applicable, employing conventional techniques such as plate counting methods and immunoassays, as well as more efficient techniques including molecular methods (e.g., polymerase chain reaction) and enzyme-linked immunosorbent assays (ELISA). Biosensor technology may also be applied in real-time to screen and detect microbial contamination of meat products (Sionek et al., 2020). Standard methods exist to detect and quantify common microbiological hazards, such as *Salmonella*, *Listeria*, and *E. coli* (U.S. Department of Agriculture, 2020a). Guidance on evaluation of viruses and mycoplasma in products derived from cell lines of animal origin is available for biotechnological products. Infectivity, electron microscopy, reverse transcriptase, antibody production tests, and in vitro assays using susceptible indicator cells may be used to detect viruses (European Medicines Agency, 1997; U.S. Food and Drug Administration, 2010). Mycoplasma can be assessed using nucleic acid amplification technique-based assays, DNA staining, and culture methods (Nübling et al., 2015; U.S. Food and Drug Administration, 2010). Although it is currently unknown whether cell-cultured product manufacturing may pose any unique microbiological hazards, no novel pathogens are expected. An evaluation of whether existing microbiological criteria for conventional meat and seafood products are applicable to cell-cultured products is warranted.

Microbiological challenge testing may be a useful approach to evaluate any potential hazards arising from

storage or food processing. Pathogenic organisms are intentionally introduced to food, then products are treated or stored under realistic conditions and analyzed for any physicochemical changes, microbiological growth, or hazardous degradation products (Komitopoulou, 2011). This testing can provide information on product stability and the effectiveness of procedures designed to eliminate pathogens.

### 4.2.2 | Residue, contaminant, and byproduct analysis

The presence of any drugs, additives, processing aids, and contaminants needs to be considered and analyzed. Although some substances are not intentionally included in the final product, residues could carry over from the manufacturing process. Limits and maximum impurity or residue levels (metals, natural toxins, agricultural or veterinary chemicals, environmental contaminants) are established for conventional livestock or aquaculture products in many jurisdictions (e.g., Food Standards Australia & New Zealand, 2021; Government of Canada, 2020). The World Health Organization (WHO) has developed a list of antimicrobials that should not be used in animals due to their critical importance for human medicine (WHO, 2019). Most antibiotic drugs currently approved for use in food animals are also approved for human use (National Research Council, 1999). It remains to be determined whether these existing criteria for conventional products require modifications or if additions are warranted for cell-cultured meats.

Companies that use novel inputs may need to develop and validate their own analytical tests to identify any residues, contaminants, or byproducts in the final product. Many chemical hazards (e.g., dissociation reagents, cryoprotectants) may be screened using conventional analytical methods such as mass spectrometry, chromatography, and immunological techniques (Chiou et al., 2015; Toldrá & Reig, 2006), though sample preparation may require modification for the cell-cultured meat matrices. Bioassays have been developed to detect a wide range of residues in conventional meat products. For example, U.S. Department of Agriculture (2011) endorses a multiple bioassay method designed to screen meat and poultry for common antibiotic groups, after which specific techniques can be used for full identification and quantification. Bioassays may also be used as a screening tool for currently unknown or unexpected hazards (e.g., migrants from equipment). Determining whether the sensitivity and range of the tests are adequate for the various inputs used in cell-cultured products, or whether the techniques will require modification, is an important topic for future research.

### 4.2.3 | Biochemical, molecular, physical, and compositional analyses

Biochemical, molecular, physical, and compositional analyses can be used as part of a comparative approach to assess the similarity to existing products. Analyses developed for safety assessment of GE food and feed and cloned animals intended for food are anticipated to apply to cell-cultured products, whether genetically modified or not (EFSA, 2008; WHO, 2008).

A molecular and biochemical analysis of cell-cultured products can help determine the extent of any differences in the genome and confirm intentional effects or identify unintentional expression of products not normally seen in meat or seafood (Sewalt et al., 2016; Stout et al., 2020). Any expression products may be compared to conventional products to identify any new or increased hazards related to consumption. Safety assessment of a GE fish, AquAdvantage salmon, and a GE pig, GalSafe pig, determined that the introduced DNA was safe for the resulting GE animal and its offspring, and that the animals are safe to eat (U.S. Food and Drug Administration, 2017a). The safety assessments relied on determining the health of the animal, as a healthy animal is likely to be safe to eat. Phenotypic characterization as well as compositional and nutritional analysis of the edible tissues was performed to ensure that there were no biologically relevant differences between the GE animals and comparator conventional animals (U.S. Food and Drug Administration, 2017b, 2020).

Methods already exist to characterize GE animals intended for use as food (WHO, 2008). As part of this analysis, the genome sequence is evaluated to determine whether the inserted genetic material changes essential gene function, to identify whether or not there are new and unintended open reading frames, and to ensure that no genes code for known toxins or antinutrients (WHO, 2008). Similarly, methods exist for biochemical and proteomic analyses to assess expression of new products and identify differences in protein, peptide, amino acid, and metabolite levels as compared to conventional meats (WHO, 2008). Any newly expressed or altered proteins may affect product stability or physical properties and alter their toxic or allergenic potential. The assessment of a novel protein may focus on amino acid sequence similarity to known toxins or allergens (e.g., Ladics et al., 2011); if significant homology is found, then further testing may be performed (e.g., stability or digestibility in the human body, toxicity testing of that protein).

The cells themselves are typically monitored throughout the process for quality control measures, which can provide an indication of cell health. For example, physicochemical properties, proliferation potential, differentiation capacity, karyotype stability, and the expression of specific

cell markers to validate identity can provide valuable safety information and identify any unwanted physicochemical transformations.

Compositional analysis is likely to be a key element of a comparative safety assessment. The analysis may include an assessment of macro- and micronutrients, bioactive compounds, toxins, and allergens. This evaluation can provide a baseline to compare cell-cultured meat with a conventional product (U.S. Food and Drug Administration, 2008; Williams, 2007); products that are similar to conventional meat are more likely to be processed and metabolized similarly and may rely on safety assessment of its conventional counterpart. A number of agencies publish specifications for meat and seafood products; these describe protein and fat content, mandatory ingredients (such as specific cuts of meat, bone, blood, organs, or skin), and optional ingredients (such as spices, filler, flavor enhancers, or water). However, even among conventional meats, composition can vary. Fatty acid profiles, for instance, can differ significantly between organic versus nonorganic products or between different muscles of the same species (Ros-Freixedes & Estany, 2014; Średnicka-Tober et al., 2016; U.S. Food and Drug Administration, 2008). Research is needed to evaluate applicability of existing specifications, characterize how cell-cultured products may differ in composition from their conventional counterparts, and determine to what extent those differences may influence safety.

### 4.2.4 | In vitro testing: Cytotoxicity and microbiome assessment

In vitro tests may be a desirable starting point for safety testing as they are more efficient and less resource intensive than in vivo testing and also avoid or reduce animal testing. In vitro testing can be used to screen for and identify potential hazards and is sometimes used to aid in dose selection for conventional animal tests. In vitro testing is typically performed on ingredients rather than whole foods, as test substances must be solubilized in media. To perform these tests on whole foods, samples would need to be freeze dried and homogenized or processed in some manner. This presents a technical challenge and likely does not accurately represent the final product. More research is required to determine whether and how in vitro safety testing can be effectively applied to whole foods. Regardless, in vitro methods may be useful for endpoint testing for any inputs to the process or novel proteins, contaminants, degradation products, metabolites, or byproducts present in the final cell-cultured product.

Beyond genotoxicity and allergenicity testing, few in vitro tests relevant to food safety have been validated for stand-alone use. Nonstandard test methods exist, such as cytotoxicity, digestion, and microbiome tests. However, these types of tests generally lack regulatory acceptance as a complete replacement for animal studies, but may be useful as supporting information as part of a broader safety assessment strategy. Cytotoxicity studies can be used as a screening tool, and may be more sensitive than in vivo tests for demonstrating safety at the cellular level. Primary cells or co-cultures of cells representing the gastrointestinal tract are used to mimic realistic scenarios (EFSA Scientific Committee, 2018; Pradhan et al., 2020). In vitro digestibility testing can analyze the stability and digestibility of foods under processing conditions (e.g., heat, freezing) or with simulated saliva and gastrointestinal fluids; the products of these conditions can then be used in toxicity tests to dose relevant cells, such as stomach cells. To determine their safety (Astwood et al., 1996; EFSA Scientific Committee, 2018; Pradhan et al., 2020).

With increased recognition of the role of the microbiome in maintaining health, in vitro assays to measure positive or negative impacts on the gut microbiome may be important to investigate. The gut microbiome is a complex ecosystem of microorganisms that support physiological, biochemical, and immunological function (McBurney et al., 2019; Roca-Saavedra et al., 2018). The presence of residues (particularly antibiotics), metabolites such as growth factors, and contaminants in food, or changes in micronutrient composition, such as vitamins, iron, and fatty acids, can alter the microbiota composition (Roca-Saavedra et al., 2018). Microbiome communities are highly diverse and individualized, and their relationship to adverse human health outcomes is still not well understood, even for conventional foods (McBurney et al., 2019). In vitro microbiome assessment for cell-cultured meat and seafood would require further research.

### 4.2.5 | Allergenicity testing

Allergenicity is a key focus of food safety assessment. For GE foods, comparative testing has been used to assess allergenicity (EFSA Scientific Committee, 2018; WHO, 2009). GM plants, such as potatoes and soy beans, have generally demonstrated similar allergenicity to their conventional counterparts (Gizzarelli et al., 2006; Lee et al., 2006), which is also expected for cell-cultured meats that are manufactured to mimic existing products; but this has not yet been demonstrated. There is also the potential to reduce the allergenicity of products, for example, through removal of alpha-gal sugars, which has been demonstrated in live pigs (U.S. Food and Drug Administration, 2020).

If any novel proteins are expressed in cell-cultured meat or seafood, in silico assessments can evaluate sequence

homology and identify structural similarities to existing proteins (Ladics et al., 2011); this characterization can help identify toxic or allergenic properties (EFSA, 2008). There are a multitude of existing allergenicity tests, including the pepsin resistance test, immunochemical cross-reactivity testing with IgE from serum of individuals known to be allergic to similar proteins, in vitro IgE-binding tests such as the radioallergosorbent test or ELISA, and skin prick testing (EFSA, 2008; U.S. Food and Drug Administration, 2015). The use of animal models to identify human sensitivity to novel allergens may not be reliable (Melo et al., 1994) or necessary unless in silico or in vitro tests indicate a need for further testing.

### 4.2.6 | Genotoxicity testing

A number of validated in vitro genotoxicity tests screen endpoints such as potential mutagenic activity, DNA strand breaks, and cytogenicity (e.g., OECD 476, 2016; OECD 490, 2016; OECD 487, 2016; OECD 473, 2016; OECD 471, 2020) and results of these tests may predict mutagenicity or carcinogenicity. This testing will be useful in identifying potentially genotoxic inputs to the manufacturing process. If it is deemed necessary to apply these tests to whole foods rather than select inputs, some of these techniques may require modification. The most common genotoxicity test, the Ames bacterial mutagenicity test (OECD 471, 2020.), for example, may not be appropriate for meats high in histidine (e.g., pork, beef, lamb, chicken, turkey, fish) because this amino acid interferes with the test (Aeschbacher et al., 1987). If a review of in vitro tests and available toxicokinetic data indicate the possibility of genotoxic effects, in vivo genotoxicity tests may be considered (e.g., OECD 486, 1997; OECD 478, 2016; OECD 489, 2016; OECD 475, 2016; OECD 474, 2016; OECD 488, 2020), though this is not expected for cell-cultured meats.

### 4.2.7 | In vivo testing

Cell-cultured meat and seafoods that are biochemically, genetically, and compositionally similar to existing foods should theoretically be as safe as their conventional counterparts. There is uncertainty as to whether in vivo testing will be required for novel inputs or products with significant differences from existing foods (e.g., because they contain potentially hazardous proteins or metabolites, or lack a conventional counterpart). In some regulatory jurisdictions, testing in rodents remains a required baseline study for novel foods, and may help assuage safety concerns. However, from an industry perspective, where the avoidance of animal use is a key tenet, the performance of in vivo testing to demonstrate safety is not desirable. Some regulatory jurisdictions promote alternative testing strategies (i.e., nonanimal testing) where possible; however, the availability and validation of reliable and representative in vitro tests that represent food consumption and mimic the human gastrointestinal tract that can fully replace in vivo testing remains a barrier and a research need.

A subchronic 90-day feeding trial (OECD 408, 2018), where rodents are fed a test substance daily for 90 days, is typically a fundamental element of ingredient safety testing. This test serves as a basis to demonstrate safety for food, feed, pharmaceuticals, agricultural products, pesticides, contaminants, and industrial chemicals. The study assesses body and organ weight, feed consumption, blood and urine chemistry, histopathology, and animal behavior to determine direct or systemic effects resulting from consuming the food. This test is generally accepted as sufficient to identify adverse effects that could occur after repeat and chronic exposure to a substance (EFSA, 2008). Ingredients or chemically defined substances such as a micronutrients or amino acids can be mixed into specially formulated diets to test in vivo safety. (Roper et al., 2019). However, animal feeding trials may not be appropriate for whole foods such as meat and seafood (EFSA, 2008; Kok et al., 2008; WHO, 2008). To improve the reliability of results obtained from animal-based toxicity testing toward the human safety context, animals are generally fed the test substance at levels that exceed those expected for human consumption of the product. However, animals may not find the food palatable, and feeding abnormally high doses of food may cause nutritional imbalances in the diet (Knudsen & Poulsen, 2007; EFSA, 2008). Therefore, the amount of whole food that can be incorporated into the test animal diet is limited by bulk and nutritional imbalance, and the detection of adverse effects resulting from any toxicants or antinutrients is likely to be missed.

Further studies may be warranted, particularly where biochemical, in silico, or in vitro tests also indicate potential concern. If genotoxicity or subchronic testing of ingredients suggests a need for chronic or carcinogenicity studies, standard tests for chronic testing (OECD 452, 2018), carcinogenicity testing (OECD 451, 2018), or combined chronic/carcinogenicity testing (OECD 453, 2018) exist. If there are any indications from subchronic studies that reproductive organs or systems involved in development may be affected, then in vivo reproductive and developmental toxicity testing may be performed. Tests such as two-generation reproductive toxicity studies (OECD 416, 2001) or prenatal developmental toxicity studies (OECD 414, 2018) may be applicable. If subchronic testing and allergy testing demonstrate possible immunotoxic effects, further investigation of the endpoints assessed in

subchronic tests may be warranted. For example, histological assessment of lymphoid organs and tissues, and hematological assessment of various cells and immunoglobulin levels may give further indication of immunotoxic effects. As with all in vivo studies, the development of alternative testing methods to effectively replace these tests is preferred and is a research priority for safety testing of cell-cultured products.

#### 4.2.8 | Human studies

For food ingredient safety assessment, a demonstration of similarity to foods that have a history of safe consumption and/or alternative testing studies and animal studies have typically been accepted as sufficient to demonstrate safety, and few foods have required human studies. Human studies may be used for whole foods, but are typically related to tasting/palatability, short-term testing for digestibility and tolerance, allergenicity, testing in support of health claims, or for specialized foods where there is a need to investigate potential negative nutritional effects or adverse health outcomes on specific populations (e.g., food for infants and children, pregnant women, patients at increased risk of a disease, etc.) (Agriculture & Agri-Food Canada, 2013; EFSA Scientific Committee, 2018).

A research gap regarding safety of foods derived from modern technology is whether recombinant DNA (rDNA) in meat is capable of transferring to microbiota in the gastrointestinal tract. In theory, horizontal gene transfer (HGT)—a process by which rDNA can pass from one species to another (e.g., to human gut microorganisms or microorganisms in the environment)—could occur. For example, antibiotic resistance genes are sometimes inserted into plants or microorganisms to distinguish GE cells during cell selection (EFSA, 2008; WHO, 2009); HGT could result in the development of populations of antibiotic-resistant organisms, reducing the effectiveness of current antibiotics (Maghari & Ardekani, 2011). Most research to date has focused on HGT potential from GE prokaryotes and plants, and has generally demonstrated that HGT events are rare, or are eventually lost due to a lack of conferred advantage (Nielsen et al., 2014; Rizzi et al., 2012). Regardless, the insertion of antibiotic resistance genes in food producing GE organisms is discouraged and even prohibited in some jurisdictions. Few studies have researched the risk of gene transfer from GE mammalian cells. Scientists have performed stability studies testing the degradation of DNA in saliva and gastrointestinal fluid, experimental studies using recipient bacteria or cells in vitro, and in vivo studies feeding animals or volunteers the sample then testing for rDNA in the body (Martín-Orúe et al., 2002; Netherwood et al., 2004; EFSA, 2008). If a cell-cultured process introduces a genetic modification, an assessment of whether the modification could introduce a fitness advantage may be warranted; this can help characterize the likelihood that the change would persist in the unlikely event of HGT.

#### 4.2.9 | Postmarketing monitoring

Postmarketing monitoring, where large populations of consumers are evaluated over the long term, has been used to complement premarket safety assessment for some novel foods (Hepburn et al., 2008; Wal et al., 2003). A postmarketing program may help detect rare and unintended adverse effects such as allergic responses. Such approaches have already been successfully applied in the medical field (Howlett et al., 2003), but it may be challenging to adapt the approach to cell-cultured products. Pharmaceuticals have well-controlled dosages, and adverse outcomes are relatively easy to track in the medical context. By contrast, it is far more difficult to monitor the adverse health effects resulting from long-term consumption of food (Hepburn et al., 2008; Howlett et al., 2003). However, pre-identification of potential hazards (such as growth factors) and tracking-related adverse outcomes may be merited. Some food manufacturers have developed monitoring systems to obtain feedback from consumers; these systems rely on various strategies such as consumer reporting of adverse effects via hot lines, performing household panels, interacting with market research companies and consumer associations, evaluating supermarket data, and engaging with medical professionals and scientific agencies (Wal et al., 2003).

## 5 | CONCLUSIONS

This review initiates the process of evaluating cell-cultured meat and seafood manufacturing processes from a risk assessment perspective by taking the first step of identifying potential hazards. Methods to manufacture cell-cultured products are not yet finalized, or at least have not yet been optimized into standard protocols; furthermore, companies may not want to publicly share intellectual property in order to protect their processes. Therefore, a generalized manufacturing diagram was developed in consultation with 87 industry representatives from 50 cell-cultured meat and seafood companies that could be applied across a wide range of production processes. In discussion with these experts, it was determined that, although many steps are common to all processes, there are also steps that are not universal, such as genetic modification. In addition, some of the process steps could be

repeated or performed in different sequences. To address these commonalities and differences, a modular diagram was developed to improve value to the industry as a whole and allow for customization by a manufacturer or safety assessor. Similarly, many potential hazards may be relevant to multiple companies, based on the manufacturing processes employed, but others will not. The identified potential hazards are linked to manufacturing activities to support a tailored approach for companies to identify and develop risk management plans specific to potential hazards related to their own processes.

Established principles and processes in related fields can help inform a safety framework for cell-cultured meat and seafood. This effort has determined that some standards and methods to assess the safety of conventional food, feed, novel foods, products of biotechnology, medicines, and other biologicals are applicable for safety assessment of cell-cultured products. Globally harmonized standard methods for quality management systems and some safety testing methods may be applicable, though there are knowledge gaps that create uncertainty. Standard approaches already exist for the assessment of food additive safety; these will likely be applicable in evaluating the safety of individual inputs, ingredients, or distinct expression products.

As the field develops, more will be learned about the types and concentrations of potentially hazardous inputs, and whether they will end up in the final product in any significant amounts, or if process steps might generate any metabolites or expression products not normally found in food. Measurement of these residues, byproducts, and contaminants in the final product, if any, would determine the degree to which their safety assessment is required. Generating standard lists that identify maximum levels of residues, byproducts, and contaminants will enable more efficient and reliable product testing. Although cell-cultured meat and seafood products may contain residues not typically found in conventional foods, contaminants that have never been identified before are unlikely. As such, it is likely that established methods will be available to address safety testing needs.

It will be particularly important to determine the extent to which cell-cultured meat and seafood products may result in differences from conventional products, where, as a result of consumption, the foods affect human health or the environment in an adverse or different way when compared to their conventional counterparts. The extent of safety testing of a final product as a whole food may depend on its biochemical, molecular, physical, and compositional similarity to foods that have already been demonstrated safe. For any product with components that may affect human health, or for those products that have no conventional comparator, a more extensive safety assessment may be required on the whole food product. However, testing whole foods with existing standard safety testing methods is challenging; these methods require a thorough evaluation for their validity to test cell-cultured foods. This also opens the door for innovation in safety demonstration for whole foods of cell-cultured meat and seafood.

## 5.1 | Research priorities

Although a number of frameworks for hazard assessment, risk management, and testing methods are applicable for safety assessment of cell-cultured meat and seafood products, gaps remain in understanding some of the novel aspects of cell-cultured meat and seafood products that may affect safety.

The following research topics address the safety information data gaps identified in this review. Many of these areas of research will require collaboration between academic, nonprofit, government, and industry groups to support development of methods that are reasonable to sufficiently demonstrate safety and to ensure that the research and outcomes are representative of realistic products. As part of the workshops, input was sought to understand the importance of these research topics from an industry perspective. Participants were asked to vote for their top priorities; these results are listed in Supplement 2.

- Evaluation of the inputs (e.g., growth factors, antibiotics, scaffold, novel inputs), as compared to conventional foods:
  - Identification of the types of inputs and concentrations used in the product;
  - Determination of where in the process the inputs are used;
  - Assessment of the efficacy of the removal steps;
  - Evaluation of the safety of the residues for use in food.
- Determination of the significance of health status of the source animal in relation to potential for disease propagation in vitro.
- Confirmation that cell-cultured products are less susceptible to contamination than conventional foods.
- Assessment of the range of genetic modification approaches and outcomes that affect safety:
  - Identification of novel metabolites or expression products;
  - Evaluation of the potential for DNA to be transferred to gut or environmental microbes;
- Development of industry-wide standards for safe residue levels of common inputs.
- Evaluation of the comparative approach for the safety assessment of the final product:

- Development of methods for comparative nutritional analysis.
- Development and validation of animal-free safety testing methods;
- Development of digestion and microbiome safety assessments with regard to inputs of concern such as growth factors.
- Assessment of whether there are any novel allergens in the final product.
- Assessment of whether media recycling concentrates hazardous inputs/residues.
- Evaluation of environmental effects of waste products and determine appropriate disposal.
- Evaluation of whether any novel food processing techniques affect safety of the final product.

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## AUTHOR CONTRIBUTIONS

Kimberly Ong: conceptualization-equal; data curation-equal; formal analysis-equal; investigation-equal; methodology-equal; project administration-equal; writing-original draft-lead; writing-review & editing-lead; Jeremiah Johnston: conceptualization-equal, data curation-equal; formal analysis-equal; funding acquisition-supporting; investigation-equal; methodology-equal; project administration-equal; writing-original draft-equal; writing-review and editing-equal. Isha Datar: conceptualization-equal; data curation-lead; formal analysis-equal; funding acquisition-lead; investigation-equal; methodology-equal; project administration-equal; writing-original draft-equal; writing-review and editing-equal. Vincent Sewalt: conceptualization-equal; formal analysis-equal; investigation-equal; writing-original draft-equal; writing-review and editing-equal. Dwayne Holmes: formal analysis-equal; investigation-equal; writing-original draft-equal; writing-review & editing-equal. Jo Anne Shatkin: conceptualization-equal; data curation-equal; formal analysis-equal; investigation-equal; methodology-equal; project administration-lead; writing-original draft-equal; writing-review and editing-Equal.

## CONFLICTS OF INTEREST

Kimberly Ong is a Senior Toxicologist and Jo Anne Shatkin is President of Vireo Advisors. Isha Datar is Executive Director and Jeremiah Johnston is Research Program Director of New Harvest. Vincent Sewalt is a full-time employee of IFF and a member of the board of directors of New Harvest. At the time of writing, Dwayne Holmes was the Head of Quality Assurance and Regulatory at Mosa Meat.

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**Contributors and workshop participants**:

Ronit Bakimer and Didier Toubia; Aleph Farms

Joshua March; Artemys Foods

Mariia Abyzova and Askar Latyshev; ArtMeat

Carrie Chan and Mario Chin; Avant Meats

Shannon Falconer; Because Animals

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Masataka Minami; NUProtein

Patricia Bubner; Orbillion Bio

Nina Buffi, Witold Maniowski, Jordi Morales-Dalmau, and Jan Saam; OSPIN Modular Bioprocessing

David Brandes and Paul Mozdziak; Peace of Meat

Ka Yi Ling and Durga Sathiakumar; Shiok Meats

Karolis Rosickas and Steve Oh; SingCell

Beth Loberant; SuperMeat

France-Emmanuelle Adil and Clément Carlier; Tiamat Sciences

George Peppou; Vow

Charles Cuerrier; Whiteboard Foods

Aryé Elfenbein; Wildtype

Two industry representatives from an undisclosed cultured meat company

## SUPPLEMENT 2: INDUSTRY-RANKED RESEARCH PRIORITIES

The research topics highlighted in this review were shared with industry experts during the third workshop to identify their priorities for future research. Participants were asked to vote on the five topics they deemed most relevant and important to address from their perspective as industry leaders. Votes were tallied in real time, and the top seven areas voted as most relevant (with percentages) were as follows:

- Develop industry-wide standards for safe residue levels of relevant inputs (68%);
- Identify the concentration of inputs (e.g., growth factors, antibiotics, scaffold, novel inputs) in the product, as a function of where in the process used and thoroughness of removal steps (60%);
- Assess whether media recycling concentrates hazardous inputs/residues (44%);
- Assess the range of genetic modification approaches and outcomes that affect safety (40%);
- Evaluate the comparative approach for the safety assessment of the final product (36%);
- Identify novel metabolites or expression products (36%);
- Evaluate whether specific food processing techniques affect safety of specific process inputs (36%).

## ORCID

*Kimberly J. Ong* https://orcid.org/0000-0001-6441-1309
*Vincent Sewalt* https://orcid.org/0000-0002-3579-3034
*Jo Anne Shatkin* https://orcid.org/0000-0001-9015-8642

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