

How Do Microbial Safety Issues Associated With Meat Apply to Cell Cultivated Products?

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FSA Research and Evidence

Cell cultivated products (CCPs) represent a rapidly emerging sector within the global food industry. These encompass a variety of foods produced through innovative processes that hold the potential to significantly enhance environmental sustainability, animal welfare and nutritional availability. Unlike traditional meat production, which involves animal slaughter and extensive farming practices, CCPs are derived from cells isolated from animals or plants, cultivated in a controlled environment and then harvested to create the final food product or food ingredient. This method enables the production of 'meat-like products' without the need for traditional agricultural methods.

Certain microorganisms (bacteria, yeast, fungi, microalgae) can also be grown in culture to produce alternative proteins. One production method is biomass fermentation (e.g., Quorn), where the whole cells are included in the food product. Another option is to use microorganisms that have been genetically modified to produce a protein of interest through a process called precision fermentation. An example of this that is currently used in the food industry is the production of chymosin, an enzyme utilised in cheese making.

The novelty of these food production techniques and the products that are being developed poses challenges for regulatory bodies like the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in conducting risk assessments based on the best available science. Microbiological hazards are a primary concern, and the aim of this project was to review available literature and seek input from experts to identify the potential hazards, sources of contamination, mitigation measures, and evidence gaps. These are presented in this report, along with recommendations that will assist in enhancing the science base, informing regulatory requirements and facilitating future microbial safety assessment of these novel products.

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Lay Summary

Cell cultivated products (CCPs) represent a rapidly emerging sector within the global food industry. These encompass a variety of foods produced through innovative processes that hold the potential to significantly enhance environmental sustainability, animal welfare and nutritional availability. Unlike traditional meat production, which involves animal slaughter and extensive farming practices, CCPs are derived from cells isolated from animals or plants, cultivated in a controlled environment and then harvested to create the final food product or food ingredient. This method enables the production of 'meat-like products' without the need for traditional agricultural methods.

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Abbreviations

AMR Antimicrobial resistance

BSA Bovine serum albumin

BSE Bovine spongiform encephalopathy

CCPs Cell cultivated products

CIP Cleaning in place

CITES Convention on International Trade in Endangered Species of Wild Fauna and Flora

DNA Deoxyribonucleic acid

EFSA European Food Safety Agency

FAO/WHO Food and agriculture organization/ World health organization

FBS Foetal bovine serum

FCS Foetal calf serum

FSA Foods Standards Agency

FSS Food Standards Scotland

GCCP Good cell culture practice

GHPs Good hygiene practice

GMO Genetically modified organism

GMP Good manufacturing practice

HACCP Hazard analysis and critical control point

HPAI Highly pathogenic avian influenza

LAL Limulus Amebocyte Lysate

PBO Precision breeding Organism

PCR Polymerase chain reaction

QbD Quality by design

rFC Recombinant factor C

RNA ribonucleic acid

SIP Steaming in place

TSE Transmissible spongiform encephalopathies

UKAS United Kingdom Accreditation Services

US FDA United States Food and Drug Administration

UV Ultraviolet

Executive summary

Alternative sources of proteins have received increasing interest in recent years since they are thought to have great potential in helping to tackle the challenge of feeding a growing global population in a more sustainable manner. Perceived benefits for environmental sustainability, animal welfare and health are key drivers of this trend. Technological advances are enabling an acceleration of innovations, and a wide range of alternative protein sources are being employed to develop novel food (and feed) products. The main categories of alternative proteins are plants, insects, algae, cell cultivated products (CCPs) and microbial proteins, which include biomass and precision fermentation production systems. Cell cultivated products encompass a variety of foods produced through innovative processes that use cells isolated from animals or plants, cultivated in a controlled environment and then harvested to create the final food product. This method enables the production of 'meat-like products' without the need for traditional agricultural methods.

Microorganisms can be used to produce alternative proteins through methods such as biomass fermentation (where the entire microbial mass constitutes the food product), and precision fermentation (where microorganisms are utilised as factories to produce the proteins of interest). These techniques allow for the creation of specific ingredients or entire foods by utilizing new microorganisms and/or new processes. While these and cell cultivated production methods are innovative, they build on established processes like brewing, the production of Quorn, and lab-based cell culture used in research or in the pharmaceutical sector. However, the novelty of these food production techniques and the products they generate poses challenges for regulatory bodies like the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in conducting risk assessments based on the best available science.

One of the primary concerns, and the focus of this research, is microbiological hazards. During production or processing, these novel foods could become contaminated with traditional pathogens. Additionally, there is a risk of novel pathogens for food emerging from the production processes themselves, including bacteria, viruses, and fungi. The identification of potential novel pathogens and the opportunity for these to grow during production and storage warrants further investigation. Microbial hazards have been identified as the first focus of

the FSA's research efforts with the aim of contributing to the emerging evidence base to support the risk assessment of cell cultivated products before they enter the food chain.

This project has combined a review of the available published literature (academic and non-academic) and an expert elicitation event bringing together a range of expertise from various relevant fields to identify the potential hazards, sources of contamination, mitigation measures, evidence gaps and recommendations that may be considered to support future microbial safety assessment of these novel products and guidance for the industry.

Key findings

Microbial hazards can be introduced at any stage of production. In CCPs, the initial phase of cell sourcing is a major risk, since the process generally involves isolating cells or tissue from an animal in a slaughterhouse. The risk of transfer of traditional foodborne pathogens that might be present in the animal to the biopsy exist. The environment of a slaughterhouse makes cross-contamination an easy occurrence, with enterobacteria as the most common pathogens. However, during cell sourcing and transport to the laboratory, high concentrations of antibiotics and antimycotics are used to ensure that any bacteria, yeast or fungi will not survive and contaminate the subsequent culture. Prions are also a concern associated with bovine/ovine sources of cells, although tissues where prions may be found in cases of infected animals are not used as source of cells, and these tissues are segregated in slaughterhouses, reducing the risk of cross-contamination. Furthermore, there is no evidence that prions can propagate in cell cultures, and they are by enlarge not considered of concern. There is also a risk of animal viruses transferring to the starting cells and contaminating the cultures. Once again, this is deemed a low risk, since animal viruses tend to be species-specific and even tissue-specific, and the likelihood of finding them in muscle or fat, where cells will be typically selected from, is low.

There are many opportunities for microbial contamination during the production process for both CCPs and microbial cultures. These include culture medium inputs, materials, equipment, water, air, operators, and a strict regime of hygiene and aseptic techniques is necessary to minimise risks. Bacteria, yeast, fungi, viruses can be introduced from these sources. Ingredients that are of animal origin, sometimes used for CCPs, pose a risk of introducing prions (as mentioned above, not consider a big risk) and viruses. Whilst most bacteria, yeast and fungi turn the culture medium turbid and thus are easy to detect in culture, viruses, mycobacteria and mycoplasma are too small and do not cause turbidity, which means that

testing would be needed to detect them. Mycoplasma are a common contaminant of animal cell cultures and are very difficult to eliminate, although their relevance to human health is not clear.

Another microbial related risk is the presence of toxins such as endotoxins, exotoxins (e.g. enterotoxins), mycotoxins and cyanotoxins, some of which can be harmful to humans at low concentrations.

Despite the opportunities for microbial contamination, since production takes place under sterility and controlled conditions, microbial risks in cultivated and microbial protein products are believed to be lower than in conventional animal-derived foods. Regarding downstream processing and manufacturing, it is likely that these stages could follow hygiene conditions like other conventional food processes.

Mitigation strategies

- Animals as source of cells for CCPs
 - Animal health inspection and veterinary certificate of health status
 - Avoid animal tissues where prions would be found in cases of infection
 - Use code of hygiene practices. Guidelines from other sectors may be helpful, e.g., clinical, biopharmaceutical industries
 - Tissue decontamination using antimicrobials and adherence to optimal conditions for storage and transportation to the laboratory
- Bovine serum and other ingredients from animal origin
 - Testing for relevant animal viruses. Early detection, before the medium component is used in culture, is critical.
 - The use of alternative non-animal derived ingredients is expected to reduce the risk of zoonotic disease greatly, although further research is needed.
- Contamination from the production environment
 - Continuous environmental monitoring (e.g., air, surfaces, water) helps to identify microbial contamination early.

- Cleanrooms and controlled environments with air handling, temperature, humidity, and particulate controls significantly reduce microbial risks.
- Contamination from operators – comprehensive training of personnel is essential. Personal hygiene, aseptic techniques, adherence to protocols are some of the practices that must be continuously reinforced.
- Testing – regular microbial testing during the production process will help early detection and avoid the spread of contamination.
- In-line real-time processing monitoring of parameters indicative of microbial growth (e.g., pH, dissolved oxygen) will help early detection of contamination.
- Sterilisation and decontamination.
- Single use technologies, such as bioreactors, tubing, filters reduce the risk of contamination, although they have a larger environmental impact.
- Close fermentation systems enable to maintain controlled conditions and minimise contamination risks.
- Regulatory guidelines and quality assurance schemes such as GMP (Good manufacturing practice), GCCP (Good cell culture practice), HACCP (Hazard analysis and critical control point) are important as they will provide a standard framework to support mitigation measures and the production of safe products.

Research needs

- Understanding the potential risk posed by animal viruses, including viruses of concern, suitable methodologies, markers of infection, role of retroviruses.
 - Understanding the full potential of real-time monitoring during production and what would be the best indicators to measure to ensure microbial safety. Artificial Intelligence and Machine Learning are being used to enhance the potential. Role of sensors for monitoring microbial metabolites.

- Improve endotoxin quantification tests to optimise performance in a wide range of novel matrices.
- Role of endogenous microbiota on microbial risks. It would be useful to understand if the lack of microflora in CCPs makes them more vulnerable to microbial contamination.
- Impact of physicochemical properties of CCPs on microbial risks and research into how microbes might be distributed within the novel products.
- Challenge experiments to understand the behaviour of potential foodborne pathogens in CCPs and novel biomass and precision fermentation products.
- Investigate impact of food processing on microbial risk- use of CCPs in raw / smoked / fermented foods.
- Establish microbial thresholds for CCPs and novel fermentation products, as they exist for conventional foods. To do this, the performance of existing methods in novel matrices must be investigated and validated, and new methods developed where needed.
- Validation of current microbiology tests with new matrices and smaller sample quantities.
- Investigate shelf-life - Meat and other animal-derived foods have a defined shelf-life, but this is still lacking for CCPs and novel fermentation products. Investigate applicability of accelerated shelf-life models to cultivated products.
- Research into microbial risks associated with alternatives to animal-derived culture medium ingredients.
- Waste from production cultures
 - Research into re-valorisation of spent medium.
 - Waste may also be a source of contamination and potentially AMR (Antimicrobial resistance) where antibiotics are used.
- As the industry continues to develop and adapt equipment and processes for CCP production, cleaning, sterilisation and other relevant procedures will require assessment and validation and national/international standards should be developed.

Recommendations

- Develop guidance and national and international standards for the industry (CCP, biomass and precision fermentation). The principles of Codex and HACCP provide a solid basis to build specific guidelines and quality control plans for this sector, and learnings can be drawn from the clinical / biopharmaceutical industry and adapted to novel food requirements.
- Research is needed to provide data that could inform regulatory requirements. Some risks can be anticipated, but there are also knowledge gaps. Specific requirements such as microbial thresholds, endotoxin limits or viral testing need to be established.
- The CCP, biomass and precision fermentation industries need methods that are validated in these novel food matrices and accredited. Further engagement with UKAS (United Kingdom Accreditation Services) to discuss options to make this process more flexible and agile would be recommended.
- Development of certified reference materials and proficiency testing (PT).
- There is a gap in the UK market in testing services for viruses. The service is limited and expensive. Acquisition and maintenance of ISO 17025 accreditation in this area is a significant factor in terms of effort and cost, and consideration should be given to improvements in this space.
- Encourage and facilitate data sharing to avoid duplicating efforts. Data comparability between experiments would be important. Development of guidance to ensure confidence and comparability of results are key, in parallel with development of national/international standards to support this and provide a framework.
- Develop a consistent regulatory framework and associated guidance to support route to market. Depending upon the product being developed, there can be ambiguity as to whether this would be treated as a GMO (Genetically modified organism), PBO (Precision breeding organism) or novel food. Also, the trade of these novel products must be regulated and associated processes clearly defined.

- Develop guidelines for waste management in this sector to minimise the potential environmental impact.

1. Introduction

Cell cultivated products (CCPs) represent a rapidly emerging sector within the global food industry. These proteins encompass a variety of foods produced through innovative processes that hold the potential to significantly enhance animal welfare, nutritional availability, and environmental sustainability. Unlike traditional meat production, which involves animal slaughter and extensive farming practices, CCPs are derived from cells isolated from animals or plants, including meat, fat, offal, seafood, or eggs. These cells are cultivated in a controlled environment and then harvested to create the final food product. This method enables the production of 'meat-like products' without the need for traditional agricultural methods.

Beyond CCPs, alternative proteins produced through methods such as biomass fermentation (e.g., Quorn), and precision fermentation (e.g., Beta-lactoglobulin), also have a role to play in the evolving UK diet. These techniques allow for the creation of specific ingredients or entire foods by utilizing new microorganisms, different feedstocks, or by inserting DNA into both new and established genetically modified microorganism (GMO) cell lines. While these food production methods are innovative, they build on established processes like brewing, the production of Quorn, and lab-based cell culture used in research or in the pharmaceutical sector. However, the novelty of these food production techniques and the products they generate poses challenges for regulatory bodies like the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in conducting risk assessments based on the best available science.

The emergence of alternative proteins, particularly CCPs, could introduce several potential hazards that need careful consideration. These include chemical hazards, nutritional disadvantage, allergens, and cell line management and microbial hazards as were detailed in the FSA hazard identification of cultivated meat published in 2023 (FSA, 2023). One of the primary concerns is microbiological hazards. During production or processing, these novel foods could become contaminated with traditional pathogens. Additionally, there is a risk of novel pathogens for food emerging from the production processes themselves, including bacteria, viruses, and fungi. The identification of potential novel pathogens and the opportunity for these to grow during production and storage warrants further investigation. Microbial hazards have been identified as the first focus of the FSA's research efforts with the aim of contributing to the emerging evidence base to support the risk assessment of cell cultivated products before they enter the food chain.

Since CCPs, biomass and precision fermentation production occur under controlled and sterile conditions, it is generally believed that the risk of zoonotic disease associated to these novel products will be reduced compared to conventional animal-derived foods (Rubio et al., 2020). In the case of cultivated meat and seafood, the absence of exposure to slaughterhouses and associated microbial hazards during production is considered a great advantage in terms of microbial risks, however, microbial contamination may occur at different points in the production system (Chriki & Hocquette, 2020; Handral et al., 2022; Ong et al., 2021). It is still unknown if these novel products will support the growth of foodborne pathogens in the same way and whether there will be any emerging risks associated with the new production systems. Therefore, there is a need for public studies into microbial risks in these products to increase our understanding of the issue and to provide evidence to support future risk assessments.

This project has combined a review of the available published literature (academic and non-academic) and an expert elicitation event bringing together a range of expertise from various relevant fields to identify the potential hazards, sources of contamination, mitigation measures, evidence gaps and recommendations. This information will support future microbial safety assessment of these novel products and the creation of suitable guidance for the industry, facilitating the authorisation of products that we can be confident are safe under the proposed uses.

2. Methodology

2.1. Literature review

A list of relevant keywords, text phrases and the date range to be used in the literature searching elements of the project was agreed with FSA, and this was shared with a network of experts from academia, research organisations and industry for any further suggestions. The resulting lists of terms are shown in Appendix A. Two sets of searches were conducted, one focused on cell cultivated production for food / feed, and a second one focused on clinical/biopharmaceutical related topics, as it was anticipated that more information might be available from the latter. The selected keywords and text phrases were used to search Web of Science Core collection. Searches were restricted to the last five years (2020 – 2024), and where number of articles retrieved was too large, only review articles were considered along with relevant articles therein. Additional focused web searches to identify science and grey literature were also completed. An initial filtering of retrieved articles was made from the titles, followed by abstract-based filtering. A total of 110 articles were selected.

2.2. Expert elicitation event

The expert elicitation event was held at Fera Science (Sand Hutton, York, UK) on 30 January 2025.

Twenty experts were invited from various professional sectors: academia, CCP industry, regulators. Eleven of them attended the event, plus five members of the Fera Science team. One additional expert provided written input.

Invited participants were provided with an event briefing based on the outputs of the literature review in advance of the event. The briefing included a list of topics to discuss grouped by categories, as follows:

1. Potential hazards

Pathogens found in conventional animal products	Mycobacteria	Yeast/fungi	Prions	Endotoxins
Bacteria	Mycoplasma	Viruses	Parasites	Mycotoxins

2. Potential sources of hazards

Source animal (biopsy)	Inputs	Media ingredients	Air, environment
Operators	Water	Materials/equipment	Storage (liquid nitrogen)

3. Stage of production

Cell/tissue sourcing	Culturing	Isolation of recombinant protein from precision fermentation	Storage/shipping
Cell banking	Harvesting	Post-harvest (manufacturing of finished product)	

4. Mitigation measures

Animal health certification	Avoid risky animal tissues (e.g., higher potential for prions)	Certified ingredients	Good practice codes
Aseptic techniques during cell bank preparations	Aseptic techniques during culturing	Avoid animal-derived ingredients	HACCP
Tissue decontamination, antibiotics, antimycotics	Reduce number of steps/manipulation	Testing and monitoring	Training or personnel

5. Research gaps

Would prions survive/propagate in culture?	Research into alternatives to animal-derived cell culture ingredients	Real-time monitoring methods
Can animal cells infected with viruses survive and propagate infection in culture?	Role of CCP microflora in microbial contamination susceptibility	Applicability of current testing methods

Would prions survive/propagate in culture?	Research into alternatives to animal-derived cell culture ingredients	Real-time monitoring methods
Chlorovirus (microalgae) found in human oropharyngeal samples - significance of this?	Role of physicochemical properties of CCPs in microbial contamination susceptibility	Data to support acceptable limits, standards, guidelines

The event briefing outlined the aims of the discussions, as follows:

1. Identify key microbial hazards in CCPs and potential mitigation measures
2. Identify knowledge gaps
3. Make recommendations

The event was conducted as a workshop, following a structured conversational process intended to facilitate open discussion and capture multiple points of view around the themes provided. The event started with preliminary information about the project and goals of the workshop. Participants were split into two subgroups, one composed of representatives of the CCP industry, and the other one including academic/regulatory representatives. Members of the Fera team were present in both subgroups, facilitating the discussion and taking notes. Two discussion sessions took place, one focused on hazards, sources and mitigations, and the second one focused on research gaps and recommendations. Each group discussion session was followed by a collective conversation to present and further discuss the key points identified by each subgroup.

The Fera team members shared and reviewed notes and produced a single document aggregating all the information and suggestions gathered in an anonymised manner. These notes were sent to participants to give them the opportunity to review and add any further comments.

Once further comments were compiled, the results were analysed and structured.

3. Findings

3.1. Findings from the literature

Microbial hazards in CCPs

The identification of potential microbial hazards associated with any foods requires an understanding of the overall production process. A recent FAO/WHO publication (FAO/WHO, 2023) considered four phases of cultivated meat / seafood production as a focus for microbial hazard identification, namely, cell selection, production, harvesting and processing and

formulation. Throughout the process, various potential sources of contamination exist, including materials, reagents, environment and operators (Pamies et al., 2022).

Cell selection or sourcing

The starter cells employed for the production of cultivated meat / seafood may be an already established cell line or cells isolated from an animal through a biopsy. When sourcing cells via a biopsy, a potential risk is the presence of zoonotic infectious agents in the source animal. However, the operating procedures are designed to minimise risks, and given that the cells are cultured in sterility, the overall risk of zoonotic disease in this context is thought to be significantly lower than in the case of conventional livestock derived foods. Contamination with bacteria, viruses, yeast, fungi, parasites (e.g., *Toxoplasma gondii*) or prions must be considered (Broucke et al., 2023). Common foodborne bacteria found in or on animals include *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria* and *Vibrio parahaemolyticus* (seafood) (Tan et al., 2024). *Brucella abortus* has also been identified as a potential hazard of relevance to CCPs (Habowski & Sant'Ana, 2024). Sources of contamination include feed, water, remaining aborted fetuses, hair, feathers, skin, environment, meat-and-bone meal, soil, sewage, bowel, respiratory track (Habowski & Sant'Ana, 2024). Animal tissue can also be contaminated with microbial toxins, such as endotoxins from common foodborne pathogens such as *E. coli*, and protein-based toxins such as botulinum toxin from *Clostridium botulinum*. These toxins pose a food safety risk and should be controlled (Manning, 2024; Tan et al., 2024).

A bigger concern is the potential presence of zoonotic viruses in the source animal (Lanzoni et al., 2022; Ong et al., 2021). These include viruses such as hepatitis A, hepatitis E and bovine leukaemia virus, which can be transmitted from infected cells to other hosts. However, it is not clear at present if infected cells from a biopsy would be able to persist in culture (Ong et al., 2021). Nevertheless, the risk can be mitigated by confirming the health status of the animal at the time of tissue biopsy and by a strict inspection of cells and tissues for any signs of infection (FAO/WHO, 2023).

The risk of introducing infectious prions from cattle may exist due to cross-species transmission through blood (Broucke et al., 2023; Hadi & Brightwell, 2021). Prions are proteinaceous infective agents that are able to replicate but do not possess genes. PrPSC is a misfolded prion protein commonly associated with transmissible spongiform encephalopathies (TSEs), and TSEs have been found to occur in cattle (BSE) deer, sheep (scrapie), cats, mink, and humans (Hadi & Brightwell, 2021). Therefore, prions should be assessed within risk management processes as a potential contaminant (Ong et al., 2024). Prions have been found in the brain, spinal cord, lymphoid tissues, tonsil, appendix, enteric nervous

system, and the blood of afflicted animals. If these tissues are avoided as sources of cells for cultivated meat and seafood, and if the source animals are certified BSE-free, the risk of prion contamination will be low (Ong et al., 2021). A recent report describing the output of EFSA (European Food Safety Agency) Scientific Colloquium 27: Cell Culture-derived Foods and Food Ingredients noted that no concerns about prions had been raised by participants, since prions are thought to be very difficult to propagate in vitro (EFSA, 2024).

Production

Cell cultures are prone to contamination by microorganisms and are therefore maintained under sterile conditions. Processing facilities, equipment, gases, operators can all be a source of contamination (Habowski & Sant'Ana, 2024). Medium ingredients may also introduce contamination, and if they are animal-derived (e.g., bovine serum), potential contaminants may include bacteria, viruses, fungi, yeast and prions, as mentioned above for the cell selection phase of the process. *Staphylococcus aureus* and *Listeria monocytogenes* are common contaminants in production facilities, the former usually derived from human skin and the latter being particularly persistent due to its ability to form biofilms from which bacteria can disperse into food (Habowski & Sant'Ana, 2024). A common problem with eukaryotic cell cultivation is contamination with mycoplasma (Broucke et al., 2023). Mycoplasma are small bacteria that can infect a range of hosts and include species that can be human pathogens. They lack cell walls, making them resistant to common antibiotics, and due to their small size, they are not easily detected by turbidity of the medium as are other bacteria. They can also bypass numerous filtration devices (even 0.2µm filters). Mycoplasma can be introduced throughout the production process including via starting materials and human intervention and can ruin the cell culture. It is estimated that ~5 to 30% of the world's cell lines are infected with mycoplasma causing important economic loss each year (Janghorban et al., 2023). Six species of mycoplasma out of around 190 species currently known, are responsible for almost 95% of cell culture contamination incidents (Fratz-Berilla et al., 2020), of which more than half are caused by *M. orale*, *M. fermentans*, and *M. hominis*. These species are found in the human oropharyngeal tract and are mainly introduced through personnel. *M. arginini* and *Acholeplasma laidlawii* are commonly isolated from foetal bovine serum (FBS) or newborn bovine serum (NBS) whereas swine trypsin is a major source of *M. hyorhinis* (Nikfarjam & Farzaneh, 2012).

In the field of biologic production, the presence of mycobacteria is also a concern. Species like *Mycobacterium tuberculosis* or *M. avium* may contaminate the production cultures and sources of contamination include the environment, biological sources of materials and operators. These bacteria have a complex cell wall that makes them very resistant and

allows them to grow across different environments. Their elimination is difficult, and their slow growth makes them challenging to detect in cell cultures. Testing for the presence of mycobacteria is a regulatory requirement for the pharmaceutical industry in Europe due to the associated human health implications (Marius & Fernandez, 2024). Some species of mycobacteria are pathogenic, and they need to be considered as potential adventitious contaminating agents in cultivated food products.

Viral contamination of cell cultures via bovine-derived sera continues to be a concern, although the situation has improved over the last two decades due to more stringent production and control measures (Broucke et al., 2023). In some cases, viruses will lyse the host cells and destroy the culture, but they may also establish sub-lethal infections that are maintained with cell passage. Infectious viruses can be released into the medium in some instances (Pamies et al., 2022). Both in the EU and USA, bovine-derived sera, regardless of country of origin, must be tested and/or treated (by heat or gamma irradiation) to ensure they are free from the following eight adventitious viruses: bovine viral diarrhoea virus, reovirus 3, rabies virus, bluetongue virus, bovine adenovirus, bovine parvovirus, and bovine respiratory syncytial virus (Hawkes, 2015). The use of such FBS (foetal bovine serum) or foetal calf serum (FCS) may also pose a risk of transmission of prions in cases where the blood originates from contaminated animals (Broucke et al., 2023).

Some production processes may use microcarriers or scaffolds to support scalability of adherent mammalian cell cultures (Bodiou et al., 2020). Microcarriers are particles that remain suspended in the medium and provide a surface for the cells to adhere to, enabling high cell density. They can be composed of different materials, included animal-derived products such as collagen. Microcarriers can be edible and form part of the finished product, or they can be temporary and require further steps for their removal or degradation (Bodiou et al., 2020). Likewise, different source materials and technologies have been investigated for scaffolds, including edible materials (e.g., collagen, fibrinogen, fibroin, plant protein isolates) and degradable biopolymers (Levi et al., 2022). The source of these materials, their production methods and any additional chemical or biological substances involved in their removal or degradation can potentially pose a risk of microbial contamination. If microcarriers or scaffolds are used, all these aspects need to be understood to conduct appropriate microbial safety assessments.

The continuous handling of the cells, sub-culturing, transferring, storage, may result in additional microbial contamination risks. Liquid nitrogen, used for cryopreservation of cell banks, is believed to be a possible route of cross-contamination and infection of cultures (Ong et al., 2021). This has also been suggested in the context of IVF laboratory techniques (Anifandis

et al., 2021). The potential for cross-contamination may be limited by storage of cell banks in the vapour phase of liquid nitrogen instead of the liquid phase. Best practice guidelines for cryogenic storage in IVF (in vitro fertilisation) settings have been outlined (Schiewe et al., 2019) and these may be transferrable to the food sector.

Regular monitoring and early detection of infection are critical to limit the occurrence of contaminations, as well as adhering to good hygiene practices (GHPs) throughout the whole production process, e.g., common equipment cleaning and sterilisation practices (FAO/WHO, 2023). The use of non-animal derived media components, e.g., from plants or recombinant expression systems, can also reduce the risk of contamination with zoonotic pathogens.

Bacterial-derived endotoxins may contaminate cell cultures through different sources, such as water, bovine serum, media ingredients/additives and equipment (Manning, 2024). Endotoxins are molecules (lipopolysaccharides being the most abundant) of the outer membranes of Gram-negative bacteria (e.g., *Escherichia coli*, *Shigella* spp., *Salmonella* spp.) that are shed during their metabolic processes. They can adhere to surfaces due to their hydrophobic nature and they are heat-stable and difficult to remove by heat sterilisation (FSA, 2023). The effect of endotoxins on cells in culture vary depending on the cell line, and the implications for CCPs in terms of consumer risks are not clear yet (Merck, 2024).

Other microbial toxins produced by bacteria or fungi may also present a risk of foodborne illness (FAO/WHO, 2023), although most likely, bacterial and fungi contamination would be easily detected (by turbidity of the medium) and the production batch discarded. Fungal spores from *Aspergillus*, *Penicillium*, and *Cladosporium* genera are widespread in the environment and can contaminate bioreactor, workspaces, and finished products (Sogore et al., 2024). Mycotoxins produced by some species of *Aspergillus* and *Penicillium* are hazardous and may pose serious health risks.

Harvesting and processing

Cell harvesting often involves washing steps that will remove a significant part of chemical and biological components of the culture. However, microbial contamination could also be introduced in this phase and harvesting methods should be designed to minimise this risk. Nonetheless, once the cells have reached this point, post-harvest contamination risks are believed to be comparable to those found in conventional food products (FAO/WHO, 2023). In a scenario where a microbial contamination (e.g., mycoplasma or viruses) was not detected during the culture phase, or a pathogen was present in an intentional input such as washing buffer, harvesting might concentrate the contaminating agent, allowing it to

potentially reach levels hazardous to consumers (FAO/WHO, 2023). Automated cell harvesting systems to replace manual harvesting could be a beneficial development to reduce the risk of contamination during the harvesting (Habowski & Sant'Ana, 2024).

The opportunities for contamination will naturally be greater in longer processes involving more manipulation. For example, if temporary microcarriers are used with cultivated cells and these need to be removed at harvest, the addition of reagents for chemical degradation would be riskier than thermal or mechanical approaches that do not involve an additional input (FAO/WHO, 2023).

Microbial hazards in biomass and precision fermentation

Beyond CCPs, alternative proteins produced by biomass fermentation and precision fermentation, also have a role to play in the evolving UK diet. Fermentation cultures can be used to grow microorganisms such as bacteria or filamentous fungi that will be harvested as biomass (e.g., Quorn), or to grow microorganisms as cell factories that produce proteins or other biomolecules that will become food or feed ingredients (precision fermentation). Although these production methods build on established processes such as brewing, their novelty in terms of techniques and food products that they generate, poses new challenges for regulatory bodies. Like in CCP production, the potential for microbial contamination is one of the risks to consider, and lessons can be learnt from the different production processes to address these common challenges.

A key microbial hazard in these production processes are toxins, which may originate from both the production microorganism and contaminants (Ritala et al., 2017). Although non-pathogenic species of bacteria such as *Bacillus subtilis* will be used for food/feed production, precaution should be taken, especially when using multispecies co-culture, to avoid bacterial strains that might produce harmful substances. An example of this is the bacterial strain *Cupriavidus necator* H16 which contains polyhydroxybutyrate in their cytoplasm (Hadi & Brightwell, 2021). The choice of microorganisms and the regulatory safety assessment can be supported by the concept of Qualified Presumption of Safety (QPS), which applies to non-pathogenic and non-toxigenic strains, many of them with a long history of safe use in the food industry (EFSA, 2021; Ong et al., 2024).

Another hazard associated with microbes used for food production is their relatively high content of nucleic acids, especially in bacteria. Nucleic acids are metabolised to uric acid, a cause of gout in humans. However, there are well-established methods for reduction of nucleic acid concentrations

in microbial products. In the case of feed applications, nucleic acids are not always a concern due to the presence of the uricase enzyme in most animals (Nyyssölä et al., 2022).

The Quorn™ mycoprotein from *Fusarium venenatum* is a good example of biomass fermentation with a long history of safe consumption. This mycoprotein underwent research and testing over many years to demonstrate safety. The fungus does not produce mycotoxins under the production conditions, and continued monitoring ensures that none are present (Ritala et al., 2017). Any new production species or changes in the culture conditions or feedstock require extensive characterisation and confirmation that mycotoxins are not being produced.

Rhizoxins can also be a safety concern in these cultures, for example, it has been shown that some strains of *Rhizopus*, like *R. microsporus*, are associated with a bacterial endosymbiont *Bulkholderia rhizoxinica*, which produces rhizoxin. This toxin may be secreted to the medium and pose a safety risk (Banach et al., 2023).

Microbial contamination may be introduced during production, and different production systems may have different risks. For example, submerged cultivation, that is frequently used for filamentous fungi cultivation, can be carried out in batch, fed batch or continuous culture systems (Barzee et al., 2022). Under continuous culture, nutrients are regularly supplied, and the cells are harvested continuously in an open system, which offers a convenient and cheaper alternative, however, the risk of contamination is higher than in batch and fed batch systems (Xie, 2022).

Fermentative microbes release compounds such as lactic acid, alcohol or acetic acid, that inhibit the growth of other microorganisms whereas they are unaffected and continue to proliferate, a phenomenon called amensalism (Teng et al., 2021). However, unhygienic practices may still lead to contamination, and spontaneous fermentation by undefined indigenous microbiota may cause the expansion of pathogenic microorganisms (Teng et al., 2021). Therefore, it is important to identify the presence of such microbes and prevent their propagation.

Precision fermentation has been used in the food industry for decades. An example of it is chymosin, an enzyme used in cheese making to replace calves' rennet as a milk coagulant and commonly produced by genetically modified *E. coli* or yeasts (Teng et al., 2021). Although there are well established approaches to ensure consumer safety for these materials, the application of the technologies to a wider range of novel products warrants consideration of any emerging safety implications. It has been suggested that the highest contamination risk for single cell proteins comes from incorrectly performed fermentation, for example, *Bacillus* spp. may form

spores if the ratio surface to volume is too low, and anaerobic spore formers can proliferate if anaerobic conditions are created in the culture (Banach et al., 2023).

Advances in engineering biology are opening new opportunities that might reduce the challenges associated with microbial contamination of fermentation cultures. An example of this is the non-model bacteria *Halomonas* spp., which has been proposed as cost-efficient production platform owing to their ability to grow at alkaline pH and high salt concentration. This reduces the risk of microbial contamination and enables production in open systems with significant cost reduction (Ye & Chen, 2021).

Microbial contamination has also been identified as a safety issue associated with microalgal food products, especially in open pond cultivation (Nyyssölä et al., 2022). Examples include filamentous cyanobacteria in chlorella tablets and pathogens like *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Aeromonas*, *Clostridium*, *Bacillus*, *Fusobacterium* and *Enterococcus* and *Clostridium* endospores in spirulina products (Hadi & Brightwell, 2021). The chlorovirus *Acanthocystis turfacea* chlorella virus 1 (ATCV-1) has been found in human oropharyngeal samples, raising concern, and suggesting that further investigation is needed to understand the relevance of the finding to microalgae production (Hadi & Brightwell, 2021).

The use of various types of waste materials as feedstock for fermented protein production may have economic and sustainability advantages, but it may be challenging from a safety perspective (Ritala et al., 2017). Microbial and chemical risks may be introduced via the growth substrate and safety records for feedstocks are required in many countries (Nyyssölä et al., 2022).

Microbial hazards associated with downstream processes and manufacturing

Following the cultivation and harvesting of the biomass, the materials are subjected to a series of processes to obtain the final product. These include treatments and addition of ingredients and additives to achieve the desired properties of nutrition, texture, flavour, smell, shape, size, etc. (Barzee et al., 2022) for product commercialisation. The risk for contamination associated with these processes is expected to be similar to those of traditional food products (Ong et al., 2023). Cell culturing needs to be carried out under sterility to reduce the risk of contamination and achieve the quality and cell density required. However, maintaining aseptic conditions post-harvest may not be feasible, mainly due to cost, so it is likely that the food processing stage could follow hygiene conditions like other conventional food processes (Ong et al., 2023). It has been pointed

out that cultivated products may lack the background microflora existing in conventional meat/fish, and this may impact on the ability of pathogens to grow in the products. The product's physicochemical properties may differ from conventional counterparts, and this may also play a role in microbial growth, influencing characteristics such as spoilage and safety. There is a need to investigate these aspects to better understand microbial risks in these products and to support risk assessments and mitigation measures, as well as suitable monitoring and testing programmes (Ong et al., 2023).

Some processing steps, e.g., heat treatment, will kill most microbial contaminants, although some spore-forming bacteria like *Bacillus* species can be a concern as the spores are heat-resistant and can withstand harsh conditions, making their removal challenging. If the spores germinate, the bacteria can grow and produce toxins leading to foodborne illness (Sogore et al., 2024).

Processing will vary greatly between types of CCP but at present, most of them are produced as ingredients and will require considerable processing to obtain the final product. De-watering, drying, texturizing and forming, flavouring, colouring and 3D food printing are just some examples of processes applied to microbial biomass (Barzee et al., 2022). Cultivated meat/seafood post-harvest processing may also include steps such as flavouring, colouring, fortification, formulation with other ingredients or 3D bioprinting for whole-cut formats. Although the application of 3D bioprinting to cultivated meat/seafood has not been thoroughly investigated yet, it is considered to carry a lower risk of microbial contamination compared to the conventional counterpart. At research scale, 3D bioprinting has been done under sterile conditions (Gurel et al., 2024). Nonetheless, effective cleaning protocols and food-grade materials should be used to avoid microbiological contamination from printer elements (Broucke et al., 2023).

Airborne fungal spores from *Aspergillus*, *Penicillium* and *Cladosporium* genera have been named as potential hazards in cultivated meat facilities. As these spores are often ubiquitous in the environment, they can contaminate work areas and tools, and they may be transferred to the finished products. *Cladosporium* contamination can cause discoloration and off-odours in cultured meat products. *Penicillium* species produce hazardous mycotoxins and *Aspergillus flavus* produces cancer-causing aflatoxins (Sogore et al., 2024). The quality of the production environments will directly influence the quality of the product. Air quality plays a critical role in aseptic environments, cleanrooms, and production areas, where microorganisms in the air are a potential hazard. The bioaerosol (microorganisms-carrying solid or liquid particles suspended in the air) in the food industry can involve many species including bacteria endospores

and exospores (e.g., *Bacillus*, *Clostridium*), vegetative cells mainly of Gram-positive bacteria (e.g., *Micrococcus*, *Staphylococcus*), moulds and yeasts (Masotti et al., 2019)

Biofilms are a particular challenge for a range of food industries. Biofilms are formed by microorganisms that adhere to a surface and grow on it enclosed within extracellular polymeric substances (EPS) which protect them against adverse environmental conditions, especially antimicrobials. The hygiene of surfaces and equipment used for food processing is critical. If cleaning and sanitising processes are not performed correctly, residues of organic and inorganic substances could remain and create a suitable environment for biofilm development. Major food pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*, can form biofilms (Marino et al., 2018). Yeast biofilms can be formed by various species, including *Candida albicans*, *Saccharomyces cerevisiae*, and *Cryptococcus neoformans* (Alonso et al., 2023). Microorganisms present in the topmost layers of the biofilm may detach from surfaces and cross-contaminate other products. This can be caused by the action of food or liquid passing over a surface, or from direct mechanical agitation.

Producers will need to have a clear understanding of the process steps and will need to establish Hazard Analysis of Critical Control Points (HACCP) plans to identify possible microbiological risks as well as chemical and physical risks. Testing/monitoring for quality assurance will be needed at each stage (starting materials, solutions/products used, decontamination procedures applied, waste disposal/recycling, etc.) (Warner, 2019). With robust HACCP plans to identify possible microbiological, chemical, and physical risks that can occur during bespoke processing steps, most risks can be suitably mitigated.

Regulatory aspects on storage, transport and handling at selling points need to be addressed as well for CCPs, both at national and international level (Bryant, 2020).

Mitigation strategies

Source of cells/tissues

The risk of introducing microbial contamination from the source animal can be minimised by using healthy animals. Although the relationship between the health status of the animal and the safety of the corresponding cell cultivated products is yet to be investigated (Ong et al., 2021), a health inspection and certification such as BSE-free are considered an important first line intervention (Broucke et al., 2023). Code of hygiene practices already exist for food production animals and animals used as source for xenotransplantation (i.e., use of live cells, tissues, or organs) and these include health screening programs (Ong et al., 2021). Some argue that prions are not able to survive *in vitro* and are not a real concern

for cultivated meat, however, until these hazards are better understood, using certified animals and avoiding tissues where prions are known to be present in afflicted animals would help to reduce any potential risks. Any tissues obtained from animals should be inspected for signs of infections and decontamination procedures should be followed (Broucke et al., 2023). The use of antibiotics may be necessary (Ong et al., 2021). When tissues/cells are transported to the laboratory, temperature and time must be controlled to minimise the growth of any potential microorganisms during that process (Habowski & Sant'Ana, 2024). A recent review (Goswami et al., 2024) gives examples of antibiotics used for oyster cell cultures and for washing tissue samples prior to culture. These include penicillin, streptomycin, and amphotericin B as the most commonly used antibiotics. The authors noted that some tissues have a higher microbial load than others and the decontamination might vary depending on this. In addition, some marine animals can carry symbiotic microbes and parasites, making it difficult to find optimal decontamination conditions.

The use of antibiotics in cell cultivated products has its drawbacks and should be minimised. There is evidence that antibiotics may have a negative impact on the performance of stem cells in culture (Broucke et al., 2023). A recent study showed that antibiotics are not required in the culture of bovine myoblasts and that higher cell growth was achieved when antibiotics were eliminated with no contamination being observed (Kolkmann et al., 2020). Nonetheless, whilst antimicrobials are used at any stages of CCP production, the presence of residues in the final product must be analysed for both, food safety and antimicrobial resistance reasons. The management of antimicrobial containing waste is an important aspect to consider, as its disposal may contribute to environmental antimicrobial pollution and development of AMR. This is at present largely unregulated, although the World Health Organization (WHO) has published its first-ever guidance on antibiotic pollution from manufacturing, which covers wastewater and solid waste management for antibiotic production (WHO, 2024). This document might be a useful reference for management of antimicrobial waste in the CCP industry.

Media components

Bovine serum is usually used in eukaryotic cell culture as it is a source of numerous nutrients and growth factors that support cell growth. The microbial hazards introduced via serum are like those potentially introduced during cell sourcing. One of the main hazards of concern is viruses. Current regulations in US and Europe establish the testing of commercial bovine serum for selected viruses, and some have proposed the use of high-throughput sequencing as a more powerful tool that enables the detection of new or emerging viruses in biological products (Paim et al., 2021). For bovine serum from countries considered to have

hazardous levels of viral contamination in cattle, sterilisation treatments are required. One such treatment is gamma irradiation, that can inactivate viruses without affecting serum functionality (Ong et al., 2024).

Another potential hazard associated with bovine serum is the presence of pathogenic and infectious prions. Stringent scrutiny has been proposed by some where bovine serum is to be used in the culture medium, and methods to remove potential prions from large volumes of medium, such as hollow fibre anion-exchange membrane chromatography, have been developed (Chou et al., 2015).

However, the industry is moving away from serum and other animal-derived media components due to ethical as well as safety concerns. Media components from sources such as plants or microbial fermentation are being developed (Ong et al., 2024). Plant hydrolysates could be an alternative to animal serum (Ho et al., 2021). Some hydrolysates tested in the biotherapeutic field have been shown to contain not only basic nutrients like amino acids, vitamins and lipids, but also beneficial peptides and growth factors that can enhance cell proliferation and protein quality. Bacterial hydrolysates from species such as *E. coli* and cyanobacteria species have also been found to be beneficial, although these may be more challenging from a food safety perspective due to the potential presence of toxins, therefore, plant and yeast hydrolysates have been proposed as a better option. Some of these have been studied in animal cell cultures but their application to cultivated food products requires further investigation (Ho et al., 2021; O'Neill et al., 2021). Other materials that have been reported for cell cultures include peptones, rapeseed cake, whey protein (Lanzoni et al., 2022), as well as hydrolysates from peas, mushrooms, yeast, and algae (Amirvaresi & Ovissipour, 2024).

The production of recombinant growth factors is considered a promising solution for cell cultivated products, and various production organisms are being explored (microbes, plants, insects) (Lanzoni et al., 2024), (<https://biobetter.bio/>), (<https://flyblast.bio/>). Nevertheless, these alternative sources of media component may have other associated risks that need to be fully understood, and tests for contamination are warranted regardless of the source (Ong et al., 2024).

Production process

In addition to mitigating microbial risks during cell sourcing and by using low-risk media components, opportunities for microbial contamination always exist, with surfaces, operators and air being major sources of contamination (Masotti et al., 2019). The risk of airborne contamination in food production is commonly mitigated by using air filters, which must be properly maintained, and positive air pressure. UV irradiation, chemical aerosolization and ozone gas are also used for air disinfection. Maintaining

aseptic conditions, avoiding recycling or re-using solutions and using online quality monitoring systems can help in reducing risks (Broucke et al., 2023). Sampling surfaces and air to evaluate the presence of microorganisms should be part of an environmental monitoring plan (EMP) to assess the effectiveness of microbial control measures, thus supporting food safety plans (Masotti et al., 2019).

Current sensor monitoring options for cultivated meat bioprocesses were reviewed recently, including sensors that monitor parameters potentially associated to microbial contamination, e.g., pH, dissolved oxygen or concentration of nutrients (Djialov et al., 2021). Janhorban and collaborators reviewed methods for the analysis of biological contaminants in biomanufacturing (Janhorban et al., 2023). One approach that has extensively been used for contamination detection in bioprocesses is Process Analytical Technology (PAT). PAT is a systematic approach to design, analyse and control biomanufacturing processes that can help prevent contamination. It allows for real-time monitoring, data analysis and adjustments to correct deviations. PAT implementation, however, can be difficult and expensive. Other tools, in addition to real-time monitoring of process parameters, include flow cytometry, polymerase chain reaction, and immunoassay techniques to detect bacterial or biological contaminants. The US FDA (United States Food and Drug Administration) established guidelines for microbial monitoring methods in pharmaceutical bioprocesses (Lowder & Whelton, 2003) but these are based on microbial growth on agar plates, so do not provide real-time data and can be time-consuming and resource intensive (Janhorban et al., 2023). The authors remarked that future advances should focus on providing cheap, sensitive real-time capabilities, with biosensors, mass spectrometry and polymerase chain reaction being mentioned as promising tools for this purpose.

Endotoxins are routinely tested in bioprocesses and the Limulus Amebocyte Lysate (LAL) assay has been the gold standard for many years. This test is extremely sensitive, rapid and easy to perform. It uses horseshoe crab blood, which contains enzymes that bind to endotoxins and cause coagulation that can be detected visually or spectrophotometrically. However, a more ethical and ecological alternative is now available, namely the recombinant Factor C (rFC). A study conducted over a period of six years compared endotoxin testing by chromogenic LAL assay with two rFC-based assays, concluding that rFC-based tests are reliable methods, equivalent or even superior to LAL assays and suitable for routine bacterial endotoxin testing (Piehler et al., 2020). Testing for endotoxins is critical due to their adverse effects on humans (Janhorban et al., 2023). Some recommend avoiding the use of Gram-negative bacteria for production of recombinant media components to mitigate the risk of endotoxins (Manning, 2024).

Although production processes for cell cultivated products will vary depending on many factors (cell lines, media, size/type of bioreactor, etc), many of the associated microbiological hazards will be common to all, and learnings can be obtained from the pharmaceutical/clinical sector, which shares the fundamental principles of *in vitro* cell cultivation. Gálvez and co-workers (Gálvez et al., 2014) designed a quality control plan to detect microbial contamination during the manufacturing of human mesenchymal stem cells for clinical applications. The plan covered the entire process from the source of cells, starting materials, reagents and intermediate products, to the final cellular medicine, and defined methods to detect microbiological contamination. The methods used included sterility test, Gram staining, detection of mycoplasma, endotoxin assay, and microbiological monitoring in process according to the European Pharmacopoeia, and each analytical technique was validated with three different cell cultures to demonstrate robustness. The authors suggested that the quality control plan would be suitable for standardisation for clinical use. As the processes for cell cultivated food products evolve and the microbial risks are better understood, this type of approach with recommended standardised methods and parameters to monitor, might become useful to support food safety in this sector.

The principles of good cell culture practice (GCCP) and good manufacturing practice (GMP) will help minimise contamination risks and ensure the safety of cell cultivated products (Lanzoni et al., 2024; Ong et al., 2021). GCCP's primary objective is to promote high standards in cell and tissue culture and to encourage greater international harmonisation and standardisation of laboratory practices, quality control systems and safety procedures. GCCP recommends keeping a detailed record of all procedures carried out to identify possible contaminants in the final product, working under aseptic conditions, avoiding the use of antibiotics and controlling the quality of culture media and other inputs. The standards set by GCCP are used in research and biopharma, but they may be prohibitive for food production. However, GCCP guidelines could be adapted to the production of cell cultivated food products (Ong et al., 2021).

GMP aims at preventing the occurrence of hazards, and it involves production practices concerning all aspects of manufacturing (appropriate design and construction of facilities, sanitary operations and maintenance, staff training, adherence to standard operating procedures, auditing, process controls) to ensure safe production of food (Ong et al., 2021). The [legislation for pharmaceutical products in the European Union](#), provides guidelines for GMP for medicinal products for human and veterinary use. The guidelines cover all steps of the life of a stem cell product, from cell isolation, cell bank establishment and maintenance, cell differentiation and expansion to the manufacturing, downstream processing, fill and finish,

storage and shipping (Nogueira et al., 2021). Practices aiming to avoid product contamination include the sterilisation of the reagents and materials, cleaning in place (CIP) whenever possible and steaming in place (SIP) to sterilise materials between batches. The same regulation permits and suggests the use of single-use components, in particular, the bioreactor parts that directly contact the cells, as disposing of them will minimise the risk of contamination. In the context of medicinal products, the concept of quality by design (QbD) can support GMP compliance (Nogueira et al., 2021). QbD is a systematic approach that aims to ensure the quality of medicines by employing statistical, analytical and risk-management methodology in the design, development and manufacturing ([European Medicines Agency](#)). One of the goals is to ensure that all sources of variability are identified, explained and managed by appropriate measures. Quality control is applied throughout the whole process, allowing for early detection of issues. All this enables the finished product to consistently meet its predefined specifications. QbD is also subjected to continuous and iterative improvement, informed by the learning from the process (Nogueira et al., 2021). Moreover, Good Hygiene Practices (GHP) are critical in the food supply including CCPs and will also assist in meeting GMP principles and avoiding microbial contamination.

Exemplar food safety plans (FSP) for cultivated seafood were published recently (Ovissipour et al., 2024) to address existing gaps in the current understanding of safety aspects of cell cultivated products and thus support the industry and regulatory agencies in ensuring the safety of these products. The article provides guidance that will help hazard identification and development of preventive controls, and two comprehensive food safety plans (based on two different scenarios) that could be easily adapted for other cultivated seafood products. This guidance was produced using systematic approaches to developing food safety plans with inputs from cell culture, pharmaceuticals, fermentation, seafood, meat, and aquaponics safety plans, and collaborating with experts from different backgrounds including the conventional and cultivated meat and seafood industries (Ovissipour et al., 2024). The plans detail interventions to be applied throughout the production process to minimise microbial contamination. These include personal hygiene, regular training and assessment of personnel, maintenance of training records, water decontamination treatment and regular testing, routine sampling at all stages of the process for microbial and mycoplasma contamination, treatment of spent media for recircularisation, aseptic techniques, packing, storing and shipping guidelines. These safety plans may be a good reference that can be adapted for application to other CCPs and fermentation scenarios.

Bioreactors

Bioreactors are large, enclosed vessels used for cell culture. They may have a media supply, a scaffolding system, an oxygenation system, a stirring mechanism, a plumbing system, depending on the type of bioreactor. There are three main groups of bioreactors based on how the medium is introduced into the main vessel: batch, fed batch, and continuous (Chandrababu & Puthumana, 2024). Fed-batch or continuous medium introduction are preferred for large volumes of cultivated cells as they also allow for automation, and recycling of conditioned medium (Allan et al., 2019).

Bioreactors and all parts that are in direct contact with the cell culture can be a source of contamination and must be sterilised if they are reusable. Single-use bioreactors are advantageous in this sense, since they provide sterile conditions without the need for complex infrastructure with steam (steam-in-place) and cleaning (clean-in-place). They use single-use bags made of high-density polyethylene films that are pre-sterilised by γ -irradiation, E-beam, or X-ray, significantly reducing contamination risks, which can be especially valuable when cultivating slow-growing cells such as stem cells (Kurt et al., 2022). Single-use bioreactors have been recommended for biopharmaceuticals by regulatory agencies to reduce the risk of product contamination, and many different systems are available (Nogueira et al., 2021).

Testing

Microbial testing plays a critical role in preventing foodborne disease, and a plethora of technologies exist that enable hazard detection. However, there is no certainty that current methods, sampling and testing regimes as currently used will be suitable for cell cultivated products (Ong et al., 2021). Current methods have been validated in conventional food matrices, and they may need to be adapted to these novel products. A current limitation for cultivated meat/seafood is the amount of sample required for standard microbiology testing, as these methods are usually based on a large quantity of the food product (e.g., 25g).

It has been shown that the natural microflora of a food product can influence its microbiological assessment, as it may interfere with the growth of pathogens (Al-Zeyara et al., 2011). There is currently no data regarding the potential native or transitory microflora of CCPs, and this will need to be assessed.

Microbial testing should be carried out throughout the culturing/growth process. Easy to use hygiene monitoring, such as ATP-testing can be used for surface monitoring. Microbial food safety testing requires in-process

testing for specific microorganisms, which will vary depending on the industry. Maintaining documentation of the supply chain will also assist in informing the testing required.

Traditional methods usually include plating and culturing for microbial identification. Rapid methods based on molecular approaches to detect microbial DNA signatures are considered alternative methods if not specifically described in standards such as ISO, or other standards (Merck, 2024). There is active research to achieve rapid microbial assessment and improve monitoring. Biosensors, spectroscopic techniques and spectral imaging combined with multivariate statistics and machine learning seem to be promising approaches (Pampoukis et al., 2022). However, emerging methodologies will need to be validated to demonstrate that they are fit-for-purpose.

Real-time monitoring systems enable early detection of contamination, preventing spreading of potential pathogens and reducing food safety risks. Artificial intelligence and machine learning algorithms can be used to analyse large datasets and recognise patterns that may be indicative of contamination, helping to predict potential contamination events and allowing for proactive measures (Janghorban et al., 2023). Disposal of contaminated batches and thorough sterilisation by heat treatment, high-pressure processing, and irradiation may be required when contamination is detected (Ong et al., 2024).

The HACCP principles have been universally applied for over 50 years to determine and mitigate food safety hazards and to develop food safety plans (Codex, 2022; Manning, 2024). Codex states that “food safety and suitability should be controlled using a science-based preventive approach”, also that food business operators should be aware of the hazards associated with their materials and processes, and that control measures that are essential to achieve an acceptable level of food safety, should be scientifically validated. Codex recommends monitoring, corrective actions, verification and documentation in relation to controls measures and the review of food hygiene systems periodically and whenever there is a significant change that might be relevant (e.g. new process, new ingredient, new product, new equipment, new scientific knowledge) to determine if modifications are needed. As frequently stated, HACCP principles will offer a solid framework to ensure the safety of CCPs, but further research is needed to address knowledge gaps and provide the science base to support appropriate safety plans for these novel products. Acceptable microbial limits have been established for conventional meat and seafood products and there are guidelines, standards, and specifications aiming to ensure consumer protection. However, for CCPs, there is a need for more public studies that could provide data to establish

similar references. Therefore, it is still unclear whether microbiological hazards in cell cultivated products will be the same as in their conventional counterparts (Ong et al., 2021).

Table 1 presents an adapted extract from (FAO/WHO, 2023) summarising the potential hazards during production of cultivated meat/seafood products.

Table 1. Summary of microbial hazards and mitigation strategies in cultivated meat/seafood (adapted from (FAO/WHO, 2023)).

Production step	Hazard agent	Problem description	Potential mitigation control measures
Cell sourcing (biopsy step)	Pathogens (bacteria, virus, fungi, parasites, protozoa), including antimicrobial resistant strains	Pathogens may be present in the biopsied tissues and eventually carried to the end product where they could be pathogenic if handled or consumed	Health certification, health inspection by certified professional, antimicrobials, maintain low temperature to minimise growth of potential pathogens
Cell sourcing (biopsy step)	Prions	Prions may be present in the biopsied tissues and eventually carried to the end product where they could be pathogenic if handled or consumed	Health certification, health inspection by certified professional, avoid tissues known to harbour prions
Cell culturing	Pathogens (bacteria, viruses, fungi, parasites, protozoa) and pathogenic agents (prions)	Pathogens in cell culture media components or other reagents may be present in the end product and could be pathogenic if handled or consumed	Following relevant good practices. Sterilization methods (heat, irradiation, filtration) can be applied depending on the composition of the media. Avoiding the use of animal-derived components. Source reagents from pathogen free regions or herds or with health certification.
Cell culturing	Pathogens (bacteria, viruses, fungi, parasites, protozoa) and pathogenic agents (prions)	Pathogenic contaminants from unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or consumed	Following relevant good practices. Aseptic handling of cells and inputs Process monitoring Antimicrobials can be used to prevent bacterial and fungal contamination Sterilization methods (heat, irradiation, filtration), if appropriate. Storage of cells in the vapor phase of liquid nitrogen.
Cell culturing	Pathogens	Cross contamination between cell lines of different origins or species may lead to the unexpected presence of pathogens, or pathogenic agents originating from the contaminating cell line	Following relevant good practices. Storage in the vapor phase of liquid nitrogen Maintaining of a data log for cell vials taken from cryostorage Regular quality checks under microscope for presence of other cells or contaminants
Cell culturing	Microbial toxins	Microbial toxins produced by	Following relevant good practices. Sterilization methods (heat,

Production step	Hazard agent	Problem description	Potential mitigation control measures
		contaminating pathogens could be carried to the end product where they could be harmful if consumed	irradiation, filtration) can be applied depending on the composition of the media Avoiding the use of animal-derived components. Avoid Gram negative species as a source of media component to avoid endotoxins. Monitoring of cells. Use of a washing procedure to remove substances. Raw material quality control.
Harvesting	Microbial toxins	Microbial toxins produced by certain microbes (bacteria/ fungi) under certain conditions can be introduced into the product during processing from equipment, ingredients, air, water, human operator can affect human health	Following relevant good practices. Responsible use of antimicrobials. Raw material quality control.
Harvesting	Pathogens (bacteria, viruses, fungi, parasites, protozoa) and pathogenic agents (prions)	Pathogens in reagents or washing media may be present in the end product and could be pathogenic if handled or consumed	Following relevant good practices. Sterilization methods (heat, irradiation, filtration) . Avoiding the use of animal-derived components. Avoid Gram negative species as a source of media component to avoid endotoxins. Monitoring of cells. Use of a washing procedure to remove substances. Raw material quality control. Cooking of end products can reduce or eliminate some pathogens.
Harvesting	Pathogens (bacteria, viruses, fungi, parasites)	Pathogenic contaminants (bacteria, viruses, fungi, parasites) due to unhygienic operators or equipment could be carried to the end product and be hazardous when handled or consumed	Following relevant good practices. Process monitoring Responsible use of antimicrobials Sterilization methods (heat, irradiation, filtration), if appropriate
Food processing	Microbial toxins	Microbial toxins produced by certain microbes (bacteria / fungi) under certain conditions can be introduced into the product during processing from equipment, ingredients, air, water, human operator If toxins are present in the final product they may lead to food-borne disease	Following relevant good practices. Responsible use of antimicrobials Raw material quality control
Food processing	Pathogens (bacteria, viruses, fungi,	Pathogenic contaminants (bacteria, viruses, fungi, parasites) due to unhygienic	Following relevant good practices. Process monitoring Responsible use of antimicrobials Sterilization methods (heat, irradiation,

Production step	Hazard agent	Problem description	Potential mitigation control measures
	parasites)	operators, ingredients, or equipment could be carried to the end product and be hazardous when handled or consumed	filtration), if appropriate

Emerging solutions

The literature consulted has identified approaches that are currently under investigation and proposed as potentially applicable to support microbial safety in CCP, biomass and precision fermentation. Some of these are listed below.

- Plasma-functionalised liquids (PFL) have been proposed for decontamination of animal tissues for cultivated meat production, as an innovative, antibiotic-free approach to ensure tissue sterility whilst maintaining cell health (Pogoda et al., 2024). PFLs are liquids that have been enriched with reactive oxygen and nitrogen species (RONS) during a plasma process at ambient conditions. These authors tried various methods and concluded that direct rinsing of porcine umbilical cord and flow dielectric barrier discharge as a source of plasma provided the best results.
- Protein Misfolding Cyclic Amplification (PMCA) technology has been proposed as a screening tool for the prions in cell culture. The application of the technique to screen for the presence of human and bovine (BSE) prions was shown in a human cell therapy product candidate (Lyon et al., 2019).
- Water has a fundamental role in the safety of fermented and cultivated food production. Water purification systems that combine reverse osmosis, ion exchange, and ultrafiltration have been suggested to remove endotoxins as well as other contaminants such as trace metals and dissolved organic compounds (Ong et al., 2024).
- It was suggested that a background microbiota (protective cultures) should be developed to be inoculated into cell cultivated meat/seafood (Habowski & Sant'Ana, 2024).
- Emerging methods such as optical and electrochemical sensors and biosensors integrated with technologies such as microfluidics are being studied for continuous and timely

monitoring of biological contaminants in biomanufacturing industries (Janghorban et al., 2023), and they may be applicable to CCP production systems.

- It has been shown that the use of ozone in both aqueous and gaseous form, can be effectively applied to reduce the risks associated with microbial biofilms. Aqueous ozone can be used for equipment sanitising. Ozonated water can cause a microbial inactivation of at least 3 log, which is considered a minimum requirement for the antimicrobial substances on biofilms (Marino et al., 2018).
- Air disinfection by cold plasma (Masotti et al., 2019). This technique is being investigated for air sterilisation and application to the food sector. It involves the passage of the air through an ionizing tube that emits high voltage discharge resulting in positively and negatively charged ions and reactive chemical species that attract and destroy airborne micro-organisms. The process generates ozone, which would need to be controlled.
- Engineering cells for the prevention of microbial contamination (Chandrababu & Puthumana, 2024). Genetic engineering can be used to enhance cell-based cultures by introducing genes for different purposes, including preventing microbial contamination, e.g., antimicrobial peptides, defence mechanisms against pathogens or biofilm minimisation.

3.2. Findings from the expert event

Discussions during the event took place first within subgroups, industry stakeholders separately from academic/regulatory experts. This helped to get an appreciation of the different concerns and viewpoints in each group, which were then shared during the general joint sessions.

Overall, industry stakeholders primarily emphasised the need for clear guidance as to which microbial parameters (and other safety parameters) they must monitor and the need for specifications that are consistent across their supply chain. They also highlighted gaps in the testing service offerings available to them, e.g., affordable viral screening, and the difficulty that some of the current food microbiology testing methods pose for the industry at their current stage of development, e.g., the large quantities of samples needed for certain accredited tests. Cell cultures are very sensitive to microbial contamination, and this means that the CCP

and microbial industries have a strong motivation to monitor and minimise microbial risks, but they would benefit from a strong regulatory framework for this sector to support food safety compliance.

Among non-industry experts, the discussions focused mainly on potential hazards and associated regulatory gaps. Some of the areas discussed where regulations need to be established include sources of cells (e.g., exotic species, live animal biopsy, dead animals from farms, supermarket meat, country of origin of cells), and the use of CCPs for food categories with specific regulations like fermented meats, shellfish ready-to-eat products, raw/smoked products. The need for data to support microbial parameters limits, method validation and accreditation and guidance for CCP and biomass and precision fermentation products was a strong point of consensus.

Microbial hazards in CCPs

- Pathogens found in conventional animal-derived products. Microbiological agents that are 'traditionally' associated with animal products such as *Salmonella* or *Campylobacter* are considered low risk for CCPs. Enteric pathogens are the main risk in slaughterhouses, and general cross-contamination from the environment can easily occur. Cells isolated from animals or their parts for cultivated meat might get contaminated with these microorganisms during cell sourcing, however, to minimise risks, concentrated antibiotics and antimycotics are used in the storage / transport solutions to ensure the sterility of the resulting cell banks. These antimicrobials are removed in the first passage of cell culture.
- Bacterial and yeast/fungal contamination would affect cell growth and turn the medium turbid, leading to disposal of the culture batch. For this reason, the risk of human pathogenic bacteria and yeast/fungi in CCPs is considered low.
- Mycobacteria. Some species of *Mycobacterium* are important zoonotic pathogens, however, the risk to humans through CCPs was stated as being very small. *Mycobacterium paratuberculosis* and *M. tuberculosis* can infect cattle, but it would not be present in muscle cells. The use of antibiotics during cell sourcing and cell bank production would minimise risks even further. Mycobacteria contamination of cultures through operators is also possible, although extremely unlikely.

- Spore forming bacteria are not deemed to be a concern during the culturing stages of cultivated meat/seafood. They would be considered later, during manufacturing, according to end product and packaging requirements.
- In the microbial biomass space, spore-forming bacteria are monitored.
- Mycoplasma is a well-known contaminant in cell cultures and bioprocessing fluids that can be introduced throughout the production process via numerous sources including materials, inputs, operators. Airborne particles and aerosols are a common vehicle of mycoplasma. The risk to humans through consumption of CCPs is considered low. Producers of cultivated meat / seafood do monitor mycoplasma contamination as it can ruin the culture. Testing is usually done either by imaging or by PCR (Polymerase chain reaction) to confirm the absence of mycoplasma.
- Prions are proteinaceous infective agents that are able to replicate but do not possess genes. In principle, the risk of introducing prions from an infected animal used as a source of cells for CCPs may exist. However, prions are not believed to be able to propagate in culture, so they are not considered a concern. Furthermore, the process of cell sourcing includes various practices aimed at minimising the risk of prions even further: (i) Only healthy animals provided with a veterinary certificate can be used to isolate cells. (ii) tissues associated with prions (such as category 3, nerve material) are not used, (iii) slaughterhouses segregate these risky tissues to avoid cross-contamination.
- Viruses: the risk of viral contamination being introduced at cell sourcing and succeeding during culture is considered very low. Potential risks will depend on the species cultured. Viruses tend to be species-specific, and even tissue-specific, and it will be unlikely to find them in muscle tissue. Low level viral contamination is likely to be diluted out in cell passage stages. However, if viral contamination occurs, it is normally difficult to detect and almost impossible to treat (Merten, 2002). Some viruses will cause cytopathic effect that can be detected by microscopy, but usually, viral infection does not cause any morphological changes in the cells and are not easily identified.

- Viruses are hard to grow in culture, which would suggest that the likelihood of viral expansion during culture would be low. Endogenous retroviruses may pose a small risk, especially in exotic species of animals that may be less well characterised than livestock species. This is because retroviral proviruses could replicate in the host genome and be transmitted through cell divisions. The risk of causing zoonotic disease is considered very low. More research is needed to better understand the risks associated with animal virus contamination of CCPs.
- As in the case of prions, cell sourcing protocols do have control points to minimise the risks of viral contamination from the source animal. Cases of viral infection presenting symptoms in the animal would not be an issue as the animal would not be certified as healthy in their documentation. A bigger concern would be cases of asymptomatic infections with zoonotic viruses, especially with low pathogenicity viruses. Enhanced monitoring and testing would be required to better control these cases. Veterinary certificates are needed for import and export to prove health status. However, viral testing is not required. The certificate would confirm that the animal is healthy and it may indicate that there is no viral infection on farm, but individual animals are not tested.
- Viral testing is not routine during CCPs production. In the pharmaceutical sector there is a list of animal viruses that must be tested for. Viral testing services in the UK are limited and highly priced. There is a gap in the UK market and some companies are using services overseas. ISO17025 accreditation required by UK supermarkets (for assurance purposes), therefore, something to consider in the future when CCPs are approved for the UK market.
- Parasites – experts commented that sometimes fish, crustaceans can have parasites, but it is very unlikely that they will be transferred during cell sourcing to the established cell lines and survive. Parasites like *Trichinella*, *Giardia* or *Cryptosporidium* have very specific life cycle requirements associated with the intestines of animals and will not survive in CCP culture conditions.
- Endotoxins. Endotoxin contamination may be introduced through the culture medium, and endotoxin testing is part of the general tests that the medium undergoes along other tests such as visual check, osmolarity, pH measurement

and general microbiology testing. Testing for endotoxins is important in CCP production as they can affect cell growth. However, it is difficult to find a good assay for endotoxin quantification and the media composition can affect the results. Some companies offer endotoxin testing but this can be expensive, and it takes too long to receive results, which can be problematic for producers.

- There is no clarity regarding maximum acceptable levels of endotoxins. In the pharmaceutical sector, there are threshold levels of endotoxin allowed in non-sterile products, but these are hard to achieve. Are the same levels appropriate for CCPs?
- Endotoxins can also be introduced via media ingredients, for example, growth factors produced as recombinant proteins in bacteria, and they can also accumulate on materials and are difficult to eliminate. Cleaning of materials / equipment and sterilisation between uses is important. Using 0.2 µm filter was discussed for adding media to cell lines but endotoxins would pass through this type of filter.
- Endotoxin contamination from the source animal is not considered a concern, as any endotoxins would be diluted down greatly during cell line preparation and expansion.
- Exotoxins, cyanotoxins, mycotoxins

Large volume of animal derived media might not dilute out some of these toxins and could be a problem.

In the case of animals as sources of cells, enterobacteria, and hence, enterotoxins, would be a possible risk. However, as mentioned above, antibiotics are used during the initial phases of cell line establishment, and bacterial growth would be visible in the culture and lead to disposal. Therefore, the risk is considered very low.

Mycotoxins, produced by fungi, can be toxic at low levels. These would need to be considered in fungal cultures, as modifications to the growth conditions might influence the ability of the fungi to produce mycotoxins.

Cyanotoxins from water are a risk – the food industry currently does not test for them. Cyanobacteria can contaminate microalgae cultures, especially if they are open air.

- Other microbial-related risks
 - Toxic secondary metabolites. Microorganisms can produce compounds other than toxins that can have adverse effect on humans. Examples of these are volatile organic compounds, short-chain fatty acids, alcohols, biogenic amines among others. These need to be considered in microbial cultures destined for food production.
 - Antimicrobial resistance. Antimicrobial residues are a problem in conventional meat production, and the potential of CCPs to contribute to the problem need to be understood. Data are needed regarding the amounts of antibiotics used in the CCP industry and how these are being disposed. Key aspects such as the availability and application of processes to eliminate antibiotics from spent media or the requirement to test for residues must be considered. An example of current treatment of animal cell culture spent medium prior to disposal is Virkon for disinfection, but this does not address the removal of antimicrobials. Where regulated compounds are used in the medium, they will need to be removed before disposal. The World Health Organization (WHO) has published its first-ever [guidance on antibiotic pollution from manufacturing](#), which covers wastewater and solid waste management for antibiotic production (WHO, 2024). This document might be a useful reference for management of antimicrobial waste in the CCP industry.
 - Microplastics might represent a risk if they contaminate CCPs as they can provide a surface for microbial biofilm formation.

Sources of microbial contamination

Cells/tissue sourcing

Hazards associated with cultivated animal cells will depend on where the cells come from (live/dead animal, tissue, embryo) and where the biopsy is taken (farm or abattoir).

There is a lack of regulation regarding cell sourcing, and this is a big risk. Cells are usually from an established cell line or from biopsy taken at slaughter. Dead animals from farms could potentially be a source of

cells, e.g., dead neonates, and some brands may prefer this type of source as they can claim no animal sacrifice has been involved. However, veterinarians will not provide a health certificate for a dead animal, so there would be an element of uncertainty in those cases. Industry partners stated that cells would never be taken from a found deceased animal, which would be higher risk, but also, dead animals are not a good source, since tissue samples need to be taken immediately after slaughter for the cells to be optimally viable.

Whilst the [Animals \(Scientific Procedures\) Act 1986](#) regulates working with animals, there is no standardised procedure to take biopsies from live animals, where samples can be taken from, etc. A live biopsy would be more complicated, would need a special veterinary, licensed premises and can only be completed on that premise. However, some producers may prefer cells from live animals as these can then be marketed as more ethical. Although the [Veterinary Surgeons Act 1966](#) does not specifically prohibit the use of live animals as a source of cells for CCPs, the subject would be subject to a range of animal welfare and ethical regulations and scientific approval. A well-defined pathway would be needed. Cross-learning from current regulations of similar processes was discussed, for example the use of horse serum for snake bites is permitted. The extraction of serum from live horses for this purpose is regulated, and it was suggested that this regulation might be a starting point to develop a suitable framework for live biopsy procedures in the CCP industry.

In abattoirs there are food-grade and non-food grade areas. Animals can only go into the food chain via abattoir, and they are only taken to slaughterhouse if their health status is certified by veterinarians.

Sources of pathogens in abattoirs include animals themselves (health status), personnel, equipment, surfaces. Enteric pathogens are the main risk in slaughterhouses as cross-contamination from the environment may occur. This can be managed through current regulations.

The complexity of the food supply chain is a factor to consider. The country of origin of the cells will often be different to the country of cell culture or where the final product will be manufactured. This trade needs to be regulated, and processes defined. Clear import controls and regulations are required.

The potential use of exotic animals as sources of cells for CCPs must be considered, including the possibility of wild-caught specimens or smuggling of bushmeat, for example. In addition to sustainability and biodiversity implications, this might constitute a route for novel microbial hazards. This could be regulated under a framework like CITES (Convention

on International Trade in Endangered Species of Wild Fauna and Flora), which restricts the species that can be consumed, but the associated hazards would need to be identified.

In principle, cells could be sourced from supermarket meat, and this could pose a risk. One of the experts indicated that they had successfully set up a cell line from supermarket meat and this may bring different hazards to those from a slaughterhouse. However, industry experts disputed this idea, arguing that good quality primary cell lines can only be successfully established from very fresh tissues.

Production process

- Bioreactors can easily get contaminated, mostly down to operator error, equipment failure or sealing of the bioreactor. Operators are not always laboratory-based personal, so they require training to sample correctly without compromising the main stock. Training on aseptic techniques is key for bioreactor handling and sampling.

Novel reactors with elements that cannot be autoclaved and are just cleaned may have higher risk of contamination. To minimise risks in these cases, more testing is completed routinely including testing of powdered media before adding to the bioreactor to ensure sterility. Robust cleaning in place (CIP) systems would be required.

- Medium ingredients and other inputs can be a source of contamination – Some ingredients may be from animal origin such as foetal / bovine calf serum (FCS / FBS), bovine serum albumin (BSA) or growth factors, and these pose a risk of zoonotic pathogens. Currently, pharma-grade medium and ingredients are largely used, although industry are finding non-animal alternatives.

The known inputs are easier to monitor, but there is much innovation in this space and the quality of the inputs is not always known. There is much research into sources of media ingredients, and it can be a complex landscape. Examples:

- plant-based options such as agricultural waste - understanding the potential for contamination is key.
- growth factors produced as recombinant proteins by microorganisms, plants or insects

- waste from yeast fermentation is being investigated for feeding mammalian cells. What would be the quality / safety parameters and thresholds for testing would need to be established.
- scaffolds might be used in CCP production, and depending in their source / nature, the hazards might be different. Some sources mentioned – plant, algae.

Any emerging risks associated with these novel inputs must be identified and evaluated.

Final product/manufacture

Existing regulations could be applied where appropriate, they could inform regulation of cultivated products. The possibility of exploring the applicability of existing regulations to CCPs was discussed, for example, raw fermented meats (more stringent regulations than raw meat), or shellfish regulations for ready-to-eat fish CCPs. This approach may avoid duplication of efforts where possible.

The manufacturing processing and the formulation of the final product may affect microbial risks. For example, smoking reduces microbial risks, hybrid products may have different risks to CCPs, depending on their composition. Risks also depend on the processing required prior to consumption, hence, whether the product is going to be eaten raw or smoked, or cooked at home before eating will need to be considered.

Controlling risks, testing and monitoring

Mechanisms to control microbial contamination risks during CCP production can be built into bioreactors. Novel designs of bioreactors should incorporate measures to control the risks. Consideration should be given to best practice applied to sampling for testing to avoid compromising sterility. Clear processes should exist establishing the required testing of cells, cabinet servicing, equipment servicing, etc to ensure that equipment is fit for the purpose. Validated methods in this space and the development/availability of validated data would be important to underpin these measures.

Bioreactors with elements that cannot be autoclaved may have higher risk of contamination. To minimise risks in these cases, more testing is completed routinely including testing of powdered media before adding to the bioreactor to ensure sterility. Validated robust cleaning in place (CIP) systems would be required.

There are real-time sensors for parameters that can be indicators of microbial contamination - pH, turbidity. Some experts expressed doubts about the need for this type of sensors, and concerns that they could

cause more issues due to dead points in the system or sensors themselves causing or leading to contamination. There would be a cost implication as well to introduce these sensors to existing reactors. It was stated that manual sampling from the bioreactors is not a problem.

Some issues related to microbial testing of cultures were discussed:

- The testing window is short during the culturing phase, and microbial testing can take a few days, which is a long time to wait for results.
- Standard sampling size in food testing is 25g. This is a large amount for CCPs. Methods need to be validated for using smaller quantities to ensure they are still fit for purpose. Sampling size also depends on the type of product. Trying to ensure a homogenous sample and sample amounts that are representative can be challenging. Spent media for testing would not be a problem, but if the biomass itself is needed, that is more limited, and homogeneity may be an issue. Spent media can be tested for bacterial contamination but testing for viruses would need biomass as viruses would be mainly inside the cells.
- Shelf-life testing was also discussed as potentially problematic due to the amount of product needed, especially where long shelf-life is anticipated.

Standards and guidelines

Due to the novelty of CCPs, there is a lack of guidelines and international standards in this industry. The biopharma sector uses national / international standards for their processes, but this would be excessive for the food industry. The general food safety standards may not be enough for cell cultivated products and there is a need for something new for this specific sector. It is difficult to know what the right balance of testing / criteria is. At present, companies start their own testing and make their own quality/safety standards. Once data are submitted to regulatory agencies, they are scrutinised, and more data may be requested.

Therefore, a well-defined framework with specifications/guidelines that are fit-for purpose and would be accepted by regulators is needed. There are ISO standards available in the clinical field which can be reviewed and adapted as a starting point. Method validation would be a key aspect to support reliable testing.

Regarding thresholds for contaminants, the microbiology side is the better defined but other parameters such as the level of endotoxin that is allowed in the final product, are not defined. Some companies have established a level but do not share this information.

Hygiene practices and measures should be clearly defined, as part of HACCP or GMP. There should be a distinction between requirements for process validation at setup, re-validation (e.g., after a failure or contamination issue) and routine monitoring. The former would require stricter measures.

Effectiveness of mitigation strategies

A summary of effective mitigation approaches based on the findings from the literature review and the expert contribution is presented below.

- Animals as source of cells for CCPs
 - Animal health inspection and veterinary certificate of health status
 - Avoid animal tissues where prions would be found in cases of infection
 - Use code of hygiene practices. Guidelines from other sectors may be helpful, e.g., clinical, biopharmaceutic industries
 - Tissue decontamination using antimicrobials and adherence to optimal conditions for storage and transportation to the laboratory
- Bovine serum and other ingredients from animal origin
 - Testing for relevant animal viruses. This is well established in the biopharmaceutical industry, where additional measures like sterilisation treatment must be applied when sourcing from high-risk geographies. Early detection, before the medium component is used in culture, is critical.
 - The use of alternative non-animal derived ingredients is expected to reduce the risk of zoonotic disease greatly, although further research is needed in this field.
- Contamination from the production environment

- Continuous environmental monitoring (e.g., air, surfaces, water) helps to identify microbial contamination early. Having an environmental monitoring plan with regular sampling and testing will also help to assess the effectiveness of any measures taken.
- Cleanrooms and controlled environments with air handling, temperature, humidity, and particulate controls significantly reduce microbial risks.
- Contamination from operators – comprehensive training of personnel is essential. Personal hygiene, aseptic techniques, adherence to protocols are some of the practices that must be continuously reinforced.
- Testing – regular microbial testing during the production process will help early detection and avoid the spread of contamination.
- In-line real-time processing monitoring of parameters indicative of microbial growth (e.g., pH, dissolved oxygen) will help early detection of contamination.
- Sterilisation and decontamination
 - Treatment of culture inputs by techniques such as heat, filtration, irradiation, chemical treatment
 - Cleaning and sterilisation of equipment, cleaning-in-place, steaming-in-place processes
- Single use technologies, such as bioreactors, tubing, filters reduce the risk of contamination, although they have a larger environmental impact.
- Close fermentation systems enable to maintain controlled conditions and minimise contamination risks.
- Regulatory guidelines and quality assurance schemes such as GMP, GCCP, HACCP are important as they will provide a standard framework to support mitigation measures and the production of safe products.

4. Research needs

This project has identified knowledge gaps and areas where further research is needed to support microbial risk assessments and mitigation strategies in CCPs, biomass fermentation and precision fermentation food products. A summary is presented in this section.

- Understanding the potential risk posed by animal viruses.
 - identify which viruses might be of concern (Hepatitis A, Hepatitis E, Avian flu (HPAI)) in the context of CCPs.
 - Next Generation Sequencing can be used for untargeted detection, but there is a need to understand when to test for unknown viruses.
 - Are there any parameters that would indicate viral infection?
 - Role of retroviruses. Viral particles or DNA, RNA should be detectable but there may be a small risk in exotic meats for retrovirus propagation within the genome. Proviruses could replicate within the host cell genome and may not be detectable.
- Understanding the full potential of real-time monitoring during production and what would be the best indicators to measure to ensure microbial safety. Artificial Intelligence and Machine Learning are being used to enhance the potential.
 - Role of sensors for monitoring microbial metabolites.
- Improve endotoxin quantification tests to optimise performance in a wide range of novel matrices.
- Investigate the impact of the lack of endogenous microbiota on microbial risks. It has been suggested that meat microflora may help control harmful pathogens. It would be useful to understand if the fact that CCPs are produced under sterility makes them more vulnerable to microbial contamination.
 - Meat microbes could help with flavour. Would there be a role for adding meat microbial populations to CCPs to achieve microbial balance as well as flavour?

- Impact of physicochemical properties of CCPs on microbial risks and research into how microbes might be distributed within the novel products. Consider how microbes might be distributed in novel products. Any risks associated with scaffolds, 3D printing, bioinks? Would bacteria be detectable within scaffolds? Would there be any hotspots, for example, in hybrid products?
- The behaviour of potential foodborne pathogens in CCPs and novel biomass and precision fermentation products has not been investigated. The ability of microorganisms to survive and proliferate depends on their environment, and challenge experiments where these novel products are inoculated with microorganisms to study their behaviour would help to better understand this question.
- Investigate impact of food processing on microbial risk- use of CCPs in raw / smoked / fermented foods.
- Establish microbial thresholds for CCPs and novel fermentation products, as they exist for conventional foods. To do this, the performance of existing methods in novel matrices must be investigated and validated, and new methods developed where needed. These data would support the creation of safety standards.
- Validation of current microbiology tests with new matrices and smaller sample quantities would help the industry, especially during the initial phase where microbial safety must be demonstrated whilst production is still small scale.
- Shelf-life - Meat and other animal-derived foods have a defined shelf-life, but this is still lacking for CCPs and novel fermentation products.
 - Test biomass at different temperatures, different times considering the conditions required for storage and shipping. Validation data looking at any trends would be important to gain knowledge in this area.
 - Accelerated shelf-life models are sometimes used to estimate shelf-life in conventional foods. Would this approach be applicable to CCPs?

- Research into microbial risks associated with alternatives to animal-derived culture medium ingredients. Some of these alternatives are expected to be lower risk in terms of microbial disease, but their safety implications must be investigated.
- Waste from production cultures
 - Research into re-valorisation of spent medium. This may be desirable to avoid wasting nutrients or to recover water, but data are needed to understand risks such as the potential concentration of toxins by dehydrating the medium.
 - Waste may also be a source of contamination and potentially AMR where antibiotics are used. Develop and validate methods to remove antimicrobials from spent medium.
- As the industry continues to develop and adapt equipment and processes for CCP production, cleaning, sterilisation and other relevant procedures will require assessment and validation and national/international standards should be developed.

5. Conclusion

The production of CCPs, precision fermentation and biomass fermentation food and feed products takes place under controlled conditions and for this reason, it is believed that their microbial safety would be greater compared to conventional foods, especially traditional animal-derived products. Existing knowledge from traditional food production can be used to anticipate potential hazards, many of which will be common to conventional and novel production systems. Current quality and safety programmes, guidelines and standards provide a good framework to start addressing microbial safety in these emerging industries. Related sectors such as biopharmaceutical manufacturing share some of the technologies and approaches used for cultivated products and can therefore inform safety strategies for the latter. However, due to the novelty of the industry, there are still knowledge gaps and further research is needed to understand microbial risks associated with cultivated products. Further scientific evidence will support the development of specific regulations and guidance for the sector. This research has identified evidence gaps in relevant topics like the behaviour and growth capacity of foodborne pathogens on these novel products, suitable thresholds of specific microorganisms, acceptable limits of endotoxins, suitability of current

testing methods, among others. Understanding these questions will help to establish suitable regulatory requirements, develop fit-for-purpose methodology and mitigation strategies, and to produce much needed guidance for industry.

Microbial risks associated with cell cultivated products, biomass and precision fermentation are not expected to be greater than those in conventional foods. Further research is needed to address some knowledge gaps and better inform future FSA risk assessments and best practice guidance for producers. However, much can also be learnt from related technologies, practices and standards used in other sectors like the clinical and biopharmaceutical industries.

6. Recommendations

- There is a clear need to develop guidance and national and international standards for the industry (CCP, biomass and precision fermentation). Producers currently are compelled to observe and assess what others are doing for guidance. The lack of clear guidance creates uncertainty regarding safety requirements, and it also results in inconsistency in the supply chain in terms of quality standards. The principles of Codex and HACCP provide a solid basis to build specific guidelines and quality control plans for this sector, and learnings can be drawn from the clinical / biopharmaceutical industry and adapted to novel food requirements. Concepts applied in those other areas such as GCCP or QbD may be applicable.
- Research is needed to provide data that can inform regulatory requirements. Some risks can be anticipated, but there are also knowledge gaps. Specific requirements such as microbial thresholds, endotoxin limits or viral testing need to be established.
- The CCP, biomass and precision fermentation industries need methods that are validated in these novel food matrices and accredited. Obtaining UKAS accreditation for new methods / matrices can be a lengthy process. A recommendation would be to consider how this process can become more flexible and agile. Further engagement with UKAS to discuss options for developing and implementing flexible scope of accreditation in this field would be helpful, as well as exploring potential learnings from systems in other jurisdictions.

- Similarly, the development of certified reference materials and proficiency testing (PT) takes time. Further engagement with established PT and reference materials providers would be recommended to ensure timely development of these analytical standards and services that will be required by accredited testing laboratories.
- There is a gap in the UK market in testing services for viruses. The service is limited and expensive. Acquisition and maintenance of ISO 17025 accreditation in this area is a significant factor in terms of effort and cost, and consideration should be given to improvements in this space.
- Data sharing is important to learn and avoid duplicating efforts. However, this may be difficult for companies due to intellectual property issues. Shared funding and academic research would facilitate this. Data comparability between experiments would also be important. Development of guidance to ensure confidence and comparability of results are key, in parallel with development of national/international standards to support this and provide a framework.
- Along with a clear and consistent regulatory framework, associated guidance to support route to market would be needed. Depending upon the product being developed, there can be ambiguity as to whether this would be treated as a GMO, PBO or novel food. Also, the trade of these novel products must be regulated and associated processes clearly defined.
- Develop guidelines for waste management in this sector to minimise the potential environmental impact of undesirable components such as antimicrobial residues (WHO, 2024).

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Appendix A. Literature search terms

Table 1. Search terms_1 (focused on food/feed CCP production)

Category 1	Category 2	Category 3	Category 4	Category 5
cell cultivated products	microbial	safety hazard	bioreactor	sterilisation
cultivated meat or fish or seafood	microorganisms	safety risk	fermenter	decontamination
cultured meat or fish or seafood	bacteria/ bacterium	contamination	media or medium	filter
lab grown meat or fish or seafood	virus OR viral	purity	ingredient	filtration
biomass	yeast		nutrient	Clean in place (CIP)
precision fermentation	fungi	HACCP	antibiotic	
mycoprotein	mould	ISO 22000	antimycotic	downstream processing
biopsy	pathogen	FSSC 22000	antifungal	viral removal
cellular agriculture	prion		scaffold	thermal processing
cell-based meat	microalgae	food poisoning	support	extrusion
lab-grown meat	toxin	microbiological risk	air	
artificial meat	mycotoxin	contaminant	oxygen	
in vitro meat	salmonella		CO2	
fermentation-derived egg protein	campylobacter		feedstock	
fermentation-derived milk protein	listeria		raw material	
Did you want to include oils and fats/ other metabolites ?	escherichia coli		bioprocess	
synthetic meat	mycoplasma		biomanufacturing	
single cell protein	beef		downstream processing	
single cells oil	pork		submerged fermentation	
bioproduction	chicken		solid state fermentation	
microbial biotechnology	quail		feedstock	
industrial	duck		growth	

Category 1	Category 2	Category 3	Category 4	Category 5
biotechnology			substrates	
microbial cell factory	lamb		growth factors	
synthetic biology	endotoxin			
engineering biology	rFC assay			
recombinant protein	LAL assay			
stem cells	Staphylococcus			
	Bacillus			
	Clostridium			

Table 1. Search terms_2 (focused on clinical / biopharma)

Category 1	Category 2	Category 3	Category 4	Category 5
Pharma	microbial	safety hazard	bioreactor	sterilisation
antibody production	pathogen	safety risk	fermenter	decontamination
IVF	virus	contamination	media or medium	filter
recombinant enzyme			ingredient	filtration
Tissue culture			nutrient	Clean in place (CIP)
Recombinant protein expression			antibiotic	
cell lines			antimycotic	
			antifungal	
			scaffold	
			support	
			air	
			oxygen	
			CO2	
			feedstock	
			raw material	
			bioprocess	
			biomanufacturing	
			downstream processing	

In search queries, terms within a category were separated by “OR”. Terms across categories were separated by “AND”. Queries were conducted with the following combinations of categories:

- a) Categories 1, 2, 3
- b) Categories 1, 2, 4
- c) Categories 1, 3, 4

d) Categories 1, 5