

Identification of hazards in meat products manufactured from cultured animal cells:

Hazards

3.1 Nutritional hazards

One of the key potential hazards highlighted in the literature was the nutritional impact of these products, as the nutrition profile of the product could be different from that it is replacing. This may arise because the meat produced in a bioreactor will not be directly identical to meat grown in an animal. At present it is impossible to fully recreate the in vivo (in life) environment in vitro (in the artificial setting) [\(footnote 1\)](#) [\(footnote 2\)](#) [\(footnote 3\)](#). There will be differences that arise between meat produced from cell lines and meat produced in animals. It is possible that this will lead to nutritional differences between cultured meat and traditional meat. The following are some considerations identified in the literature:

- There may be some vitamins, minerals and other nutritionally relevant components that are present in meat that may not be present in cultured meat. Examples included vitamin B1277, creatin [\(footnote 4\)](#), carnosine, vitamin D3, and iron [\(footnote 5\)](#), which are not created in muscle cells but are transported to the cells from elsewhere in the body or from the diet [\(footnote 6\)](#) [\(footnote 7\)](#).
- The proposed solution for absence of key components would be to add vitamins and minerals to the culture [\(footnote 8\)](#), but it is yet to be evaluated whether this will be taken up into the final product and compared in bioavailability to traditional meat [\(footnote 9\)](#).
- There are other factors relevant to meat that are not produced in the cells themselves but are important to the development/composition of muscle [\(footnote 10\)](#), e.g. myoglobin [\(footnote 11\)](#), haemoglobin [\(footnote 12\)](#), blood, extra/cellular tissue, lymph [\(footnote 13\)](#), and fat cells [\(footnote 14\)](#). These components which contribute nutritionally to meat may not be present in the final product.
- Muscle from an animal is highly complex as it is made up of over 6500 different proteins in different fractions that contain different muscle fibre types [\(footnote 15\)](#). It is yet to be fully evaluated whether growing the cells in the ex vivo culture environment fully replicate the protein composition of the meat it may replace.
- Cells are grown on a media formulation which provides the nutrition when culturing [\(footnote 16\)](#) [\(footnote 17\)](#). This nutrition formulation may directly impact the final composition of the cells and may produce a difference in composition to the traditionally produced meat.
- Cells require stimulation [\(footnote 18\)](#) [\(footnote 19\)](#) and simulation of the natural environment [\(footnote 20\)](#) in a bioreactor to stimulate growth [\(footnote 21\)](#). This stimulation through use of hormones [\(footnote 22\)](#), growth factors [\(footnote 23\)](#) [\(footnote 24\)](#), cytokines [\(footnote 25\)](#), nutrition [\(footnote 26\)](#) [\(footnote 27\)](#), 3D scaffold materials [\(footnote 28\)](#), bioreactor environment [\(footnote 29\)](#) [\(footnote 30\)](#) will not be identical to the environment in the host animal, so differences could arise between the final composition of the products. This is compounded as these signals are often delivered in regulated ways, across gradients and through stem cell niches [\(footnote 31\)](#) [\(footnote 32\)](#), which helps impart their effects and control the cell growth. Whilst in the production of cultured meat these will be delivered in a bioreactor in a

homogeneous cultured broth mix or with different concentration gradients, lacking the complexity of biochemical/physical regulation found in the body.

- Maturation is needed to turn nascent muscle fibres into fully formed fibres ([footnote 33](#)). This process may not be fully replicated in the ex vivo culture environment ([footnote 34](#)), and the quality of the protein may not match that of traditional meat.
 - There may be extra components that are required for the bioprocess that are not required for cultured meat that could contribute to the nutrition or the antinutritional properties of the final product ([footnote 35](#)).
1. The cells are adherent in nature and need to be grown on a scaffold ([footnote 36](#)) ([footnote 37](#)) ([footnote 38](#)). This scaffold could be part of the final structure of the product. The additional nutrition that a scaffold may provide or take away from the meat needs to be accounted for ([footnote 39](#)).
 2. The scaffold material may not be present in a proportion similar to traditional meat, with one paper stating that it may account for 25% of the total product ([footnote 40](#)). Therefore, the scaffold may contribute more significantly to the nutrition of the meat than the extra cellular matrix would for traditional meat, changing the final composition of the cultured meat.
 3. If there is a nutritional deficit or antinutritional factors in the scaffold, then this may be passed on to the consumer. One author noted that collagen is often used for the generation of cultured meat scaffolds, and this is high in the amino acid glycine ([footnote 41](#)) that may result in side effects to the consumer.
 4. In some cases, the scaffold material does not form part of the final product, however the cells are detached from the scaffold ([footnote 42](#)). It is possible that during this process e.g. mechanical, physical, biological, separations, could damage the cells/protein, lowering the quality of the final product ([footnote 43](#)).
 5. The chemical components used in the scaffolds may transfer into the final product. For instance, calcium and sodium have been used in casting methods ([footnote 44](#)) ([footnote 45](#)), and this may transfer into the product, increasing the presence of these components.
 6. Currently, cultured meat lacks the sensory and nutritional properties of traditional meat. Therefore, additives, such as flavourings, colourings, myoglobin, vitamins, and minerals may be added to the culture. Their impact on the final nutrition of the product will need consideration ([footnote 46](#)) ([footnote 47](#)) ([footnote 48](#)).
- A risk may be generated around the promissory narrative of cultured meat ([footnote 49](#)), with proponents purporting it to be healthier through design e.g. controlling the amount of polyunsaturated fatty acids ([footnote 50](#)) ([footnote 51](#)) ([footnote 52](#)) when it may not be healthier depending on the exact specification of the final product formulation. This may lead to consumers over consuming or consuming it when it is not much healthier, and this could have a negative nutritional impact.
 - Further to the previous point, there will be limits to how far the meat can be designed to have specific properties, because there will be sensory limitations on what can be added, and saturated fats are those associated with increased flavour versus polyunsaturated fats ([footnote 53](#)).

3.2 Contamination from components used in cell culturing

There are several separate stages of development for producing cultured meat and at each stage, different chemicals, biologics, media formulations, additives and supplements are used to ensure a successful culture. The contamination risk of each input needs to be assessed, as any undesirable components that remain in the final product need to be at an acceptable exposure level or need to be food-grade/food safe. Below are some considerations that were found in the literature:

- Different components can/are used throughout cell culturing (see Table 2). These compounds could be absorbed by the cell or become associated with the cell wall and

could be transferred into the final product [\(footnote 54\)](#) [\(footnote 55\)](#) [\(footnote 56\)](#). An understanding of how these compounds could bioaccumulate/be present in the final product and how they are removed or are at an acceptable exposure level needs consideration.

- Scaffold materials are used to produce cultured meat [\(footnote 57\)](#) [\(footnote 58\)](#) [\(footnote 59\)](#). Under the conditions of culturing, it is possible for the scaffolds to fracture/break, degrade, leave residuals [\(footnote 60\)](#) [\(footnote 61\)](#) potentially contaminating the final product with undesirable fragments, or leaving trace amounts that need to be accounted for (i.e. how does the scaffold degrade, are these degradation products safe, does detachment from the scaffold leave any residuals, how are these removed/accounted for).
- Antibiotics and fungicides [\(footnote 62\)](#), such as penicillin, streptomycin and gentamicin are commonly used in cell cultures to prevent infection [\(footnote 63\)](#). Although culturing can occur without the use of antibiotics/fungicides [\(footnote 64\)](#), it is likely that they will be used to prevent infection of the culture at some stage in the manufacturing process. The risk of exposure to antibiotics and fungicides residues needs to be understood.
- Some cell isolation, proliferation and differentiation protocols use toxic/non-edible/dangerous chemicals that would be toxic to humans. One example, is the chemicals that are used to differentiate pre-adipocytes into adipocytes, dexamethasone (steroid), indomethacin, toxic xanthine and IBMX [\(footnote 65\)](#), which would not be allowed in food products [\(footnote 66\)](#).
- Culturing occurs under sterile/aseptic conditions to prevent contamination of the culture [\(footnote 67\)](#). Many plastic components are used, e.g. plastic flasks, pipettes, sterile wrapping, cleaning spray bottles, and single use bioreactors [\(footnote 68\)](#). Plastics can produce toxic leachables and inhibitory chemicals into cell lines [\(footnote 69\)](#). It needs to be understood if any toxic or inhibitory leachables are present in the final product at an unacceptable exposure level. Bioreactors and all equipment are stringently sterilised and cleaned e.g. with heated steam, using chemicals like sodium hydroxide and 70% ethanol solution [\(footnote 70\)](#). Residual chemicals left over from cleaning could transfer into the final product if not properly managed.
- Throughout the cell culturing process, the cells may come into contact with metal components, namely the bioreactor, pipes, pump etc [\(footnote 71\)](#). The possibility that heavy metals could leach into the final product needs consideration.
- Biological components derived from animals and bacteria can transfer disease into the culture. Serum (e.g. foetal bovine serum) traditionally added to cell cultures poses a zoonosis risk through virus transfer [\(footnote 72\)](#). Animal derived collagen is often used as the scaffold material [\(footnote 73\)](#). Most cultured meat companies are now using serum free mediums and working on non-animal derived scaffold components [\(footnote 74\)](#). However, components derived from bacterial cultures e.g. growth factors and scaffold materials may also pose a risk of bacterial contamination [\(footnote 75\)](#). The risk of disease transfer from components derived from animals or bacterial sources needs to be understood.
- Chemical contaminants could still potentially be present in the cell culture due to a lack of quality control process, such as using non-deionized or filtered water, or not using filtered air or CO₂, but this would likely result in a spoiled culture medium rather than posing a risk to the consumer. HACCP and quality control processes will be needed to demonstrate safety.
- The cells used in culturing are metabolically active and it could be possible for them to produce metabolites or other components that could contribute negatively to the final product [\(footnote 76\)](#). An understanding of the cell metabolism and a production and removal of unwanted compounds should be demonstrated.
- There may be chemical/physical/biologics components that are intentionally added to the culture that may need to be removed from the final product. Examples include non-biodegraded and non-edible scaffold materials, such as polystyrene, polyacrylamide, polyethylene-glycol, cross-linked dextran etc, and enzymes that are added during the detachment process [\(footnote 77\)](#).

Table 2: Potential components that may be used in cultured meat production (footnote 78)

Component type	Potential Components
Components to control the proliferation, differentiation, maturation of the cells	Growth Additives, Growth Hormones, Steroids, Sodium Benzoate, Collagen powder, Xanthan gum, Mannitol, Cochineal, Omega-3-fatty acids, Bone Morphogenic Proteins, transforming growth factor β , Zinc-finger protein Zfp423 Transcription Factors, Myokines, Adipokines, cytokines, interleukin-1, interleukin-6, interleukin-10, hepatocyte growth factor, and tumour necrosis factor alpha, MYOD, MRF4, TGF1, Testosterone, Progesterone, MYOD, muscle specific regulatory factors,
Growth Media components and components added to keep the cells alive/provide nutrition	Glucose, Amino Acids, Vitamins, Minerals, Buffers, Serum Medium, Serum Free Media, Growth Factors, Binding Proteins, Adhesion Factors, Vitamins, Hormones, Mineral Trace Elements, Oxygen Carriers, Modified Haemoglobin, Artificially produced perfluorochemicals, Perfluorochemicals, FBS, HS, L-glutamine, High Glucose, E2, TBA, TBA-E2, amino acids, inorganic salts, buffer systems, carbohydrates, antibiotics, supplements, basal medium, of Dulbecco's Modified Eagle Medium (DMEM), GlutaMAXc, inhibitors and activators of cellular pathways e.g. p38 inhibitor SB203580, poloxamers,
Scaffolds Materials used to support the growth of the cells by providing material to grow on	Collagen, Collagen Balls, Collagen Mesh, Coating materials, Extra Cellular Matrix Proteins, Laminin, Cellulose, Chitosan, Collagen, polyethylene, polystyrene and epoxy, poly(glycolic acid), poly(lactic-co-glycolic acid), polylactic-acid and poly(N-isopropyl acrylamide), gelatine, fibrin, Matrigel and elastin as hyaluronic acid, chitosan, agar, dextran, or alginate, Cytodex, RGD Biding groups, RHO-associated protein kinase inhibitors, fibronectin,
Bioreactor Components/Cleaning Chemicals	Anti-foaming agents, anti-coagulation agents, thinning agents, thickening agents, cleaning chemicals, sterilisation chemicals, NaOH, Defoamers, Emulsifiers, surfactants, the material of the bioreactor itself e.g. metal, plastic.

3.3 Cell Culture Infections

Cell lines can become infected with adventitious agents (i.e. microorganisms that may have been unintentionally introduced into the culture system product) that can impact the performance of the culture or spoil the culture. These include contamination with other cells, bacteria, yeasts, fungus, viruses, and mycoplasmas, as well as contamination/cross contamination with other cell lines (footnote 79).

3.3.1. Bacterial, yeast and fungal infections

- Infection (sometimes referred to as contamination) can be caused when a fast-growing microbe dominates by using up the resources and outgrowing the selected cell line (footnote 80). Infection is visible by microscope or by eye due to turbidity and a rancid smell. The contamination may arise from contaminated reagents, contaminated air, the water bath, poorly cleaned/maintained equipment, the cell culturist, not following cleaning protocols and not following good laboratory practices (GLP) and good manufacturing practices (GMP) (footnote 81). In cell culture, these infections are generally controlled with the growth antibiotics, fungicides, etc (footnote 82).
- Rapid action must be taken to protect neighbouring culture flasks/bioreactors and prevent the infection from spreading (footnote 83). Strict quality control and critical control measures must be enacted to destroy all infected cells by ensuring clean and sterile worksurfaces. Corrective action e.g. retraining of operators, cleaning of equipment, better monitoring must be put in place. As this infection is rapid an infected batch should be discarded to reduce the risk to consumer (footnote 84).

3.3.2. Mycoplasma infections

- Mycoplasma are small, gram-negative bacteria that do not possess cell walls and are not affected by many antibiotics (footnote 85). Around 20 different species have been identified from cell culture (footnote 86). They can evade the filtering processes, such as filtering the cells over 0.22 μ m filters. These cells do not cause turbidity in the culture but impact the culture by slowing cellular growth/changing growth rates (footnote 87), altering the cell metabolism (footnote 88), physiology and causing chromosomal aberrations (footnote 89). Data from the literature suggest that somewhere between 5%-35% of cell lines are infected

with some form of mycoplasma infection, either with a single species of mycoplasma or more than one species ([footnote 90](#)) ([footnote 91](#)).

- Contamination from Mycoplasma could arise from contaminated reagents, air, poorly cleaned/maintained equipment, not following good laboratory and manufacturing practices ([footnote 92](#)). Cell lines should be regularly screened to check for mycoplasma infections ([footnote 93](#)).
- Mycoplasmas are not visible by eye or microscope and are harder to detect. Infections can be detected using testing methods such as DNA staining, fluorescent staining and PCR detection ([footnote 94](#)).
- The most common practices (if mycoplasma infection is found) is to discard the cells using autoclaving, incineration or disinfecting and discarding following protocols ([footnote 95](#)). In rare cases, the infected cell line can be decontaminated, but this is a difficult and uncommon practice and should only be completed by an experienced operator ([footnote 96](#)).
- The significance of mycoplasma infection to cultured meat is still to be evaluated, as mycoplasma infections impact the performance of biomedical research and analysis, but it has not been assessed whether this poses any risk to the consumer. It is known that there are over 200 different types of mycoplasma infections, with the majority being harmless, but there are a few that can cause infections in humans ([footnote 97](#)). These include:
 1. Mycoplasma pneumoniae
 2. Mycoplasma genitalium
 3. Mycoplasma hominis
 4. Urea plasma urealyticum
 5. Urea plasma parvum

3.3.3 Viral contamination

- Viruses are small biological entities that can infect a culture. Due to their small sizes, viruses cannot be spotted with the naked eye or standard laboratory microscopes ([footnote 98](#)). This makes them hard to spot and a challenge to remove from reagents and the cells they originate from ([footnote 99](#)). However, if they are only Cytopathic (morphological), they may have no impact on the cell culture ([footnote 100](#)). There are different more costly tactics to remove/inactivate viruses such as retention in an acidic or basic environment, or through using small nano/micro filters ([footnote 101](#)).
- Viral contamination can originate from the donor organisms originally used to extract the cell source or can originate from animal sourced components like serum media, feeder cells and other derived components ([footnote 102](#)). They can also arise through other routes of contamination common to cell culture e.g. poor adherence to GMP processes and lack of/not following the proper aseptic techniques ([footnote 103](#)).
- It is not evident or considered in the literature whether viruses will pose a significant health hazard to humans or animals through consumption of cell cultured meat products, but some literature sources suggest that the risk will be less or tantamount to that already posed by eating meat ([footnote 104](#)).
- With regards to the spreading of viruses to humans from cultured meat, most viruses are host specific, limiting their ability to cross contaminate cell lines, although they can mutate and infect different hosts ([footnote 105](#)). However, if exotic animals become more common place in the diet through the cultured meat process, there may be a risk that an unknown virus harboured in a host species could transfer from the cell culture to humans.

3.3.4. Endotoxin infections

Endotoxins are hydrophobic, heat-stable lipopolysaccharides that can contaminate laboratory equipment and become present in the cell culture. Due to their hydrophobic nature they can become stuck to contaminated laboratory equipment such as plastic stirrers and transfer into

cellular culture, with sources of contamination coming from impurified water, laboratory equipment, from media, reagents, serum components and recombinant proteins made in *E. coli*. Due to their heat-stable nature, they cannot be destroyed using heat sterilization processes [\(footnote 106\)](#) [\(footnote 107\)](#). There is evidence that endotoxins can produce variability in cell culturing results [\(footnote 108\)](#). Whether endotoxins need to be considered as a risk for cultured meat is an uncertainty, as they were not considered in the literature empirically [\(footnote 109\)](#) [\(footnote 110\)](#) [\(footnote 111\)](#) [\(footnote 112\)](#) [\(footnote 113\)](#) [\(footnote 114\)](#) [\(footnote 115\)](#).

3.3.5. Cross-contamination/misidentification issues

- There may be a risk of cross contamination of one cell line into another cell due to the use of multiple cell lines. This is a problem that came to fore in the 1960's when HeLa cells were identified in a number of cell lines. This is currently still present 18% to above 36% of cell lines showing some contamination, with a number of studies still showing varying results for the percentage of cells that have become cross contaminated/misidentified. One cell culturing bank/company reported that up to 18% of their cell lines were cross contaminated with another reporting 25% of cross contamination. The problem is quite widespread with one report stating that there were 32,755 articles reporting on cross-contaminated cell lines [\(footnote 116\)](#) [\(footnote 117\)](#) [\(footnote 118\)](#) [\(footnote 119\)](#) [\(footnote 120\)](#).
- The cross-contamination of cell lines has had a negative impact in terms of biomedical research and other cell culture applications [\(footnote 121\)](#). This has often led to many results being invalidated due falsification of results, incorrect research being completed and inaccurate results, but the real impact cannot be known.
- The cross-contamination of cell lines can come from many sources, primarily due to failure to follow good manufacturing and laboratory practices. This can include poor maintenance of equipment, poor cleaning regimes, incorrect storage of cells, poor use/cleaning of equipment, working with multiple cell lines in one area, using the wrong cells, incorrect labelling of cells, a drop of cells accidentally transferring from one flask to the next, etc. [\(footnote 122\)](#) [\(footnote 123\)](#). Good manufacturing practices therefore need to be followed to ensure that cell lines do not become mixed and miss-identified.
- Furthermore, identity testing should be part of the process to ensure that any cell lines used are authenticated. There are many methods available for authenticating cells and these include PCR based methods, karyotyping the cell line, genetic sequencing by short tandem repeat and isoenzyme analysis. Cell culture operators should be working to ensure that any cell lines purchased should come from reputable cell banks that can supply certificates of authenticity of the cell line [\(footnote 124\)](#) [\(footnote 125\)](#) [\(footnote 126\)](#) [\(footnote 127\)](#).

3.4. Cell line associated risks

The stem cells and progenitors' cells that can be used to produce cultured meat are very complex and nuanced. Forecasting specific potential hazards that may arise due to use of different cell lines or cell line specific risks are hard to make, as each company is likely to be working with their own cell line, which will have its own specific risk considerations [\(footnote 128\)](#). There are a few generalities and considerations that are associated with using cell lines:

- One potential hazard, or perhaps a quality control issue, is whether there is enough control over the process to correctly differentiate and mature cells into a final product comparable to traditional meat [\(footnote 129\)](#).
1. The cells need to be proliferated, differentiated and matured into the final product, such as a muscle fibre, but due to the differences between ex vivo processing and the in vivo growth of muscle in an animal, the final composition of the cultured meat could be different when compared to its in vivo counterpart [\(footnote 130\)](#).
 2. Meat undergoes a number of processing stages before it reaches the shop, including being slaughtered, bled and aged, but for cultured meat there are no slaughtering process

([footnote 131](#)) and may be no aging process (will be producer-specific choice), which impact the composition and quality of the meat, and will likely create differences between cultured meat and traditional meat ([footnote 132](#)).

3. Even if the cells may have correctly differentiated, the maturation of the cells may be incomplete, and the cells may not have the composition that would be expected for an in vivo muscle fibre ([footnote 133](#)). This may not be an issue, depending on the target nutritional profile, but this information needs to be readily available, and no false claims made in marketing the final product.
 - Another potential hazard is that of cells not properly differentiating, either producing cells of inadequate quality, or producing cells that are of a different cell type, in some cases forming of carcinomas/cancer cells ([footnote 134](#)). This could lead to undesired cells being in the final product.
 - There needs to be some form of quality check, to ensure that desired differentiation and maturation occurs and that the cells that make up the final product are authenticated/checked, such as using biomarkers to track differentiation, or using genetic techniques ([footnote 135](#)) ([footnote 136](#)) ([footnote 137](#)).
 - When using cell lines, but with special regards to immortalized cell lines, induced pluripotent stem cell lines and embryonic cell lines, the cells will mutate over time and be subject to genetic and phenotypic drift ([footnote 138](#)) ([footnote 139](#)). After several subculturing/passaging the cell lines may be divergent from the original cell line sequence, and the cells may express a different cellular morphology and behaviour. This may pose a risk, and a quality control for the number of times cells can be passaged/doubled may be needed.
 - One other area is the potential to use cell lines of animals not commonly eaten in the diets of those countries ([footnote 140](#)) ([footnote 141](#)). The chance to eat exotic animals may be appealing to the consumer, but as the cell lines could be generated for a range of animals there may be risks of eating new types of meat, such as transfer of new diseases and viruses (if cell lines are not properly vetted and authentic), as well as potential allergic reactions to new proteins.
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