FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk assessment – Application A1269

Cultured quail as a novel food

Executive summary

Vow Group Pty Ltd (Vow) submitted an application to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of cultured quail cells as a novel food. The cultured quail cells are made with embryonic fibroblasts originating from *Coturnix japonica* (Japanese quail).

In assessing this application, FSANZ had regard to a recent Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) publication titled Food Safety Aspects of Cell-Based Food (FAO/WHO 2023). This publication discussed potential hazards in the four stages of cell-based food production: 1) cell-sourcing; 2) cell growth and production; 3) cell harvesting; and 4) food processing.

FSANZ's hazard and risk assessment focussed on the first three stages and considered potential hazards associated with the cell line, the novel production process (limited to Vow's current scale of production and including any relevant inputs used to grow and propagate the cultured quail cells), and the cells after harvesting, which includes collection, packaging and freezing (harvested cells).

The harvested cells are a main ingredient that will be mixed with other permitted food ingredients to produce a final mixed food product and served at a maximum of 300 g of the harvested cells per serve per day. It is Vow's responsibility to ensure that any additional ingredients used in the formulation of the final mixed food comply with any relevant requirements in the Code.

Cell line

The cells used by Vow were originally isolated from a Japanese quail embryo and immortalised as an embryonic fibroblast cell line, designated by Vow as cell line 221523-Fib-Quail. Immortalisation ensures the cells can proliferate indefinitely under appropriate culture conditions. Vow provided evidence to confirm the species of the cells as well as the genetic stability of the cell line during the production process. Some genetic variation arising from the immortalisation and culturing process was identified but is consistent with what would be expected for cultured cells and does not itself raise any specific food safety concerns. Vow provided evidence that the source farm for the quail cells was under an official monitoring

regime and that tests for specific avian pathogenic bacteria, viruses and mycoplasma were negative.

Method of production

Vow confirmed that all materials used in the production process meet the requirements for food grade or pharmaceutical grade ingredients with a purity and quality suitable for their intended use in food. The processing conditions are designed for food production following Hazard Analysis and Critical Control Point (HACCP) principles supported by good practices such as Good Cell Culture Practice (GCCP), Good Hygienic Practice (GHP) and Good Manufacturing Practice (GMP). The production process in this application consists of preparation and maintenance of cell banks (master and working), cell expansion (seed train) and cell harvesting. Currently no independent microbiological data or specifications exist against which to assess the hazards of this particular food. It is also still a new area and no criteria have been established internationally. However, adopting a HACCP-based system supported by good practices in the production of the cell biomass as a food will limit the potential for ingress of foodborne pathogens during the cell expansion phase.

Harvested cells

While there is no history of consumption of cultured quail cells as food there is a long history of safe consumption of quail meat and eggs. Evaluation of the basal media and other inputs used during the production process demonstrated there are no safety concerns from exposure to these substances from consumption of the harvested cells. The available information indicates the harvested cells are unlikely to pose a food allergenicity concern for the general population. Vow analysed for the presence of gluten in the harvested cells due to the potential carry over of barley proteins from the cell culture medium. Levels were below the limit of detection of the assay used.

Vow has undertaken a preliminary microbiological analysis of the harvested cells which formed the basis for the microbiological hazard assessment. Given the aseptic nature of cell proliferation/biomass production stages, the main microbiological risks occur during harvest and post-harvest handling. This step is where harvested cells are exposed to the food production environment and any foodborne pathogens therein, particularly *Listeria monocytogenes*. Harvested cells have not undergone any microbiological control step during production other than use of good practices to prevent entry into the culture. A recognised mitigation step (e.g. cooking) before consumption would ensure safety of the final food, particularly as a safeguard for vulnerable persons. The likelihood of microbiological hazards entering the cell biomass post-harvest has been assessed at the current scale of production. As production scale increases and multiple draw down and media top up processes occur, opportunities for microbiological contamination also increase. This highlights the importance of effective implementation of hazard management through good practices at all stages of production and processing.

A nutrition risk assessment and dietary intake/exposure assessment was conducted to determine if the consumption of the harvested cells would cause a nutritional imbalance in the diet. No nutritional issues were identified for the majority of nutrients assessed. More detailed evaluations were undertaken for some specific nutrients found to be present at high levels. These were cobalamin, biotin, folate, iron and sodium. The levels of cobalamin and biotin in the harvested cells resulted in intakes that were up to 929 times the estimated average requirement (EAR) and 9 times the adequate intake (AI) respectively per serving, however no upper levels (UL) have been set for these vitamins and no adverse effects have been reported from their high consumption. Similarly, a folic acid content per 300 g serving of harvested cells may exceed the UL in individuals aged 14-18, if total folate is present as folic acid. However, this is not expected to be of concern based on the likely overestimation

of serving size and expected infrequent consumption of harvested cells.

The concentrations of iron and sodium in the harvested cells were higher than chicken breast. The total high intake of iron did not exceed the UL for all the Australian and New Zealand population subgroups assessed, even if consumers eat 300 g of the harvested cells daily in addition to other conventional meats. At this consumption level of harvested cells, the increase in the dietary intake of sodium, compared to high baseline usual intake, ranged from 8% to 19% for the Australian population aged 2-3 years, however a 300 g serving size is likely to be an overestimation for this age group.

Conclusions

FSANZ has undertaken a hazard and risk assessment of cultured quail cells, derived from cell line 221523-Fib-Quail, taking into account microbiology, biotechnology, toxicology, nutrition and dietary intake/exposure considerations.

The cell line is genetically stable and microbiological hazards associated with cell line sourcing are very low. There are no safety concerns from exposure to the substances used in the production process at the estimated consumption levels.

The harvested cells are unlikely to pose a food allergenicity concern for the general population. Vow analysed for the presence of gluten in the harvested cells due to potential carry over of barley proteins from the cell culture medium. Levels were below the limit of detection.

Microbiological safety of cultured quail cells can be assured through the systematic assessment and management of hazards throughout their production, from point of cell sourcing onwards. Food businesses producing cell-cultured food must verify changes to their processes so hazards are identified and remain effectively managed.

There were no nutritional risks identified from the consumption of the harvested cells containing the levels of nutrients provided in the application, particularly given the likely infrequent consumption of the harvested cells.

No new scientific information was received in response to the 1st call for submissions that would change the conclusions of the hazard and risk assessment presented therein. Minor updates were made to this supporting document, which are identified at Appendix V.

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List of terms

Defined below are a number of terms used specifically by FSANZ in relation to this application and in the context of this supporting document. FSANZ use of these terms may differ to how they are used by others outside of FSANZ.

Basal media	Media used to supply essential nutrients (e.g. vitamins, salts, sugars) to the cells during their growth in culture. No animal-derived components are used. Provided by a third party.
Clean-in-place (CIP)	Method of cleaning and sterilising the interior surfaces of equipment (e.g. pipes, bioreactors, tanks, filters) without disassembly.
Conventional meat	Farm-raised meat (e.g. beef, pork, lamb, chicken, quail, etc.) or game meat.
Conventional quail	Farm-raised bird.
Culture media	Media used in the production of Vow's cultured quail cells, which includes basal media and other inputs.
current Good Manufacturing Practice (cGMP)	Current GMP as defined by US FDA (2022) CFR 117 Sub-part B Clause 10 – 110.
Embryonic fibroblast	A fibroblast that is derived from an embryo. Fibroblasts are a specialised cell type that are essential to the structure of tissues and body organs.
Gene Ontology	A computational framework for representing the functions of genes and gene products based on their molecular function, cellular component, and biological processes. This is useful for bioinformatic analysis.
Good Cell Culture Practice (GCCP)	A set of principles to support best practice in cell and tissue culture.
Good Hygienic Practice (GHP)	A set of principles and procedures used in food preparation environments that when followed minimises the risk of food contamination.
Good Manufacturing Practice (GMP)	A set of principles and procedures that when followed ensures that the food produced is safe and suitable for consumers.
Growth factors	A class of polypeptides or proteins that play important roles in the regulation of cell division and tissue growth in an organism. They are often used in cell culture to promote the proliferation of cells.
Harvested cells	Cultured quail cells after harvesting.

1 Introduction

Vow Group Pty Ltd (Vow) submitted an application to FSANZ to amend the Australia New Zealand Food Standards Code (the Code) to allow the use of Vow cultured quail cells as a novel food ingredient in food products to be marketed and sold in Australia and New Zealand. The cultured quail cells are made with embryonic fibroblasts originating from *Coturnix japonica* (Japanese quail).

In assessing cultured quail cells as a new food, FSANZ has taken a step-wise approach that was informed by the outcomes of a recent Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) led Expert Consultation on Food Safety Aspects of Cell-Based Food (FAO/WHO 2023). Potential hazards were discussed in the context of four stages of cell-based food production: (1) cell-sourcing; (2) cell growth and production; (3) cell harvesting; and (4) food processing. Experts participating in this expert consultation agreed that, while many hazards are already well known and exist equally in conventionally produced food, the focus may need to be put on the specific materials, inputs, ingredients (including potential allergens), processes and processing equipment that are unique to cell culture based food production.

FSANZ's risk assessment was restricted to the first three stages of cell-based food production (Figure 1) and focused on identification of potential hazards associated with the:

- cell line, designated by Vow as cell line 221523-Fib-Quail;
- novel production process (limited to Vow's current scale of production), including any relevant inputs used to grow and propagate the cells; and
- harvested cells.

The final stage (food processing) was not assessed from a production perspective but it is understood the cells once harvested, frozen and rethawed, will be used as the main ingredient in a final mixed food product and served for human consumption at a maximum serve of 300 g of the harvested cells per serve per day.

One of the critical inputs in the production process is the basal media, including the growth factors (GFs) used to support the growth of cells in culture. The assessment considered two GF combinations that were used in the basal media during the production process: GF 1/GF 2a and GF 1/GF 2b. The identity of these GFs is Confidential Commercial Information (CCI) therefore cannot be disclosed in this report. Further information can be found in Sections 3.1.3 and 3.2.

The harvested cells will be mixed with other permitted food ingredients (e.g. calcium chloride, microbial transglutaminase, oil, textured vegetable protein, etc.) to form a final mixed food product such as a log, roll or patty (Figure 1). This stage has not been assessed by FSANZ. It is Vow's responsibility to ensure that any additional ingredients used in the formulation of the final mixed food product comply with the Code, including to maintain its microbiologically sound status.

In addition to the GFs, some information relevant to this assessment is also CCI, so full details cannot be provided in this public report.

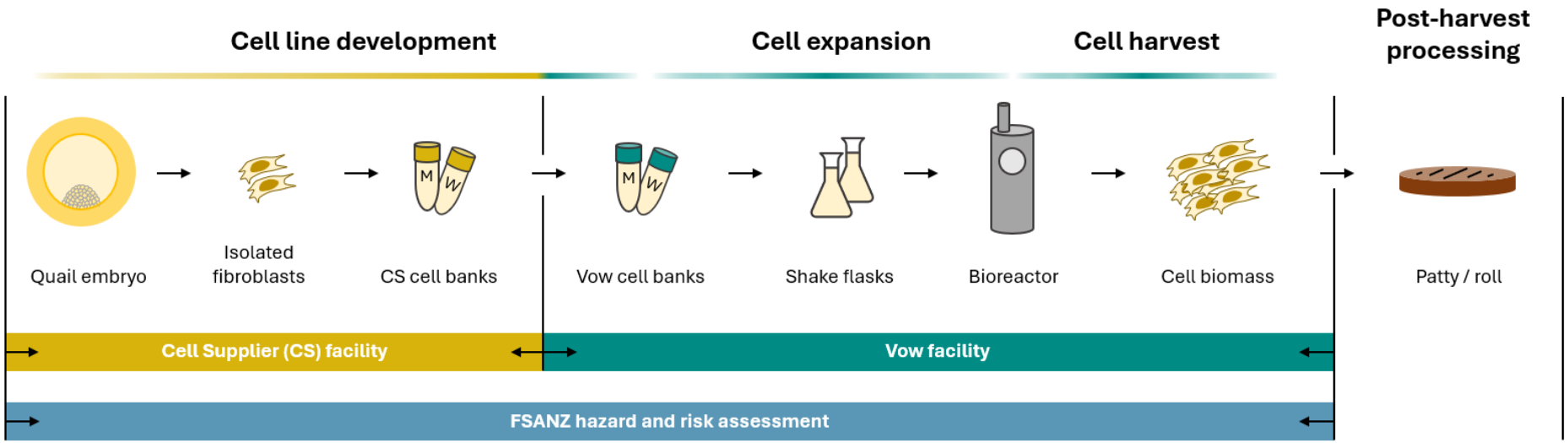

Figure 1 *A schematic overview of the production of cultured quail cells and the scope of FSANZ's assessment. M = Master cell bank. W = Working cell bank*

2 Cell line

Vow cultured quail relies on an animal cell line that can be propagated repeatedly and produce new batches of harvested cells for food use. A number of different abbreviations are used to describe the cell line at different stages of the development and production process. These are described in Table 1.

Table 1 *Cell line abbreviations used in this report*

Abbreviation	Description
csMCB	Cell line supplier Master Cell Bank
csWCB	Cell line supplier Working Cell Bank
vMCB	Vow Master Cell Bank
vWCB	Vow Working Cell Bank
vCQ	Vow Cultured Quail (at harvest)

2.1 Cell identity

The cell line is derived from Japanese quail (*C. japonica*). Primary cells were isolated from a quail embryo and immortalised as a fibroblast cell line (Figure 1). Immortalisation of the cell line ensures the embryonic fibroblasts can proliferate indefinitely under appropriate culture conditions.

Immortalisation occurred from spontaneous genetic variation as the cells were serially sub-cultured with reduced serum[1] and in suspension. Cells were then adapted to grow in serum-free suspension growth medium. Vow has assigned this cell line the unique identifier of 221523-Fib-Quail.

Embryonic fibroblasts typically have the potential to differentiate into different cell types (Yusuf et al. 2013; Singhal et al. 2015). This is dependent on the characteristics of the cell line and in response to certain culture conditions (Dastagir et al. 2014). Vow has specified that fibroblasts are the only cell type that make up their cultured quail cells, i.e. the cells retain their identity in culture as fibroblasts.

2.1.1 Species confirmation

Vow provided DNA barcoding data generated by external laboratories to validate the species of the cells used in the production process. The mitochondrial cytochrome C oxidase subunit 1 (COI) gene sequence was used as a marker to identify the species (Cooper et al. 2007; Hebert et al. 2003). Polymerase chain reaction (PCR) was used to isolate the COI gene sequence from genomic DNA extracted from the csMCB and vMCB. PCR products were sequenced and compared to online databases[2]. The analysis confirmed the cells in csMCB and vMCB were from the *C. japonica* species.

[1] Serum is used in culture media to provide cells with a variety of growth and survival factors, buffering agents and nutrients to support healthy growth *in vitro*.
[2] Barcode of Life Data System (http://www.boldsystems.org/) and/or BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

2.2 Cell line hazard assessment

In considering the safety of cell-cultured food it is important to identify potential hazards associated with the cell line. Any identified hazards then become the focus for further assessment, including whether there is any risk associated with these hazards under the intended conditions of use.

The focus of this section is to identify hazards that are associated with the development of the cell line and its proliferation in culture. This includes the vertical transmission of microbiological hazards, cell line stability and any known hazards specifically associated with quail.

2.2.1 Vertical transmission of microbiological hazards

A summary of the microbiological hazard identification and description, including expected mitigation and the information provided is in Appendix A4.1. A microbiological safety assessment was performed by the cell line supplier as part of the establishment of a cell line for use by a laboratory or by the pharmaceutical industry in accordance with international guidelines for use in vaccine development (US FDA cGMP – 21 CFR 2022; European Pharmacopeia 2008; ICH 2022). This includes identification of host-specific intracellular pathogens of avian species, including viruses, *Mycoplasma* spp. and bacteria that could impact the quality and use of the cell lines for their original intended purpose in the pharmaceutical industry. This assessment includes health of the animal, housing environment, and testing of the cell lines following primary isolation and immortalisation.

The microbiological risk depends on the species origin of the cell line. The closer the genetic relationship of the cell line to humans, the higher the risk. Given known species-specific transmission barriers exist, avian and invertebrate cells are considered to be the lowest risk groups (Pauwels et al. 2007; Herman and Pauwels 2014; Weiskirchen et al. 2023). However, where a zoonotic pathogen is known to infect humans, the usual transmission route from animal reservoir to human would need to be incorporated into the assessment (e.g. H9N2 influenzae transmission typically occurs via direct contact with infected birds rather than via a food route). Further microbiological testing may be done by the cell line user to ensure it meets their specific requirements which would depend on the end use.

The focus of the microbiological testing by the cell line supplier was on pathogens that represent an animal health issue and which have the potential to impact the culturability of the cells and thereby their usefulness for pharmaceutical and research use. Vow completed similar testing on the cell line when establishing the vMCB and vWCB.

In Australia, the importation of a cell line for the purposes of laboratory or food end uses is also assessed under the Biosecurity Act 2015 by the Department of Agriculture, Fisheries and Forestry (DAFF).

2.2.1.1 Source animal

Conventional quail can be infected with and harbour various microbiological organisms with zoonotic potential including viruses (Makarova et al. 2003), foodborne bacteria (Erdogrul 2004), and parasites (Cong et al. 2017a; Cong et al. 2017b). Prion diseases have been reported in mammals but not in birds (Kim et al. 2022), which is believed to be due to structural differences in the prion protein (Myers et al. 2020; Pietropaolo et al. 2008).

Developing cell lines from healthy animals is key to managing potential zoonotic risk of foodborne pathogens from the initial phase of the cell culturing process. The health status of the source animal could influence the safety of cell-cultured food through the potential for a microbiological hazard to enter the cell line at the point of acquiring the primary cells (FAO/WHO 2023; FSA 2023). Potential contamination of a cell line from the source animal can be reduced by veterinary examination and quarantine of animals intended to be donors, and banking of multiple cell lines to reduce the number of animals to be biopsied (Merten 2002). The relationship between the health status of the source animal, the likelihood of introducing microbiological hazards to cell lines, and severity of consequences to human health through consumption of cultured cell products is an area that would benefit from further research to better understand the level of risk.

There is less risk where primary avian cells are isolated from inside eggs as embryonic cells. Only microbial pathogens that were present in the adult bird, that infected the reproductive system and able to be vertically transmitted could potentially be present in the subsequent cultured quail cell line.

Vow provided evidence, in the form of an official veterinary certificate, verifying the farm where the quail eggs were sourced was subject to official monitoring. According to the certificate, no animal diseases or animal epidemics were officially identified in the quail breeding facility. No increased losses in the stock were documented by the owner that would indicate an endemic infection. Additionally, a microbiological examination of the eggs from the egg packing station in 2018 did not reveal any evidence of *Salmonella*. The cell line supplier (forming the csMCB/csWCB) concluded the source animals in the facility were healthy and free from disease, and therefore their eggs were suitable as donors to establish a primary cell line. As part of meeting Australian biosecurity requirements, the cell line supplier provided declarations the cell lines had no signs of contamination, including cytopathic effects, with adventitious infectious agents or microbial contamination. They also declared the cell line and/or avian derived media used to support the cell line have been sourced from animals free from diseases of biosecurity concern (such as avian influenza, Newcastle disease and virulent infectious bursal disease), or the cell line and/or media was tested and found free of these pathogens. Vow applies the cell bank concept (vMCB and vWCB) that reduces the number of cell aliquots to be sourced from the cell line supplier and tests the cell line for Influenza Type A, Newcastle Disease virus, *Chlamydiacae* spp. and *Mycoplasma* spp.

2.2.1.2 Isolation and development of cell banks

Direct infection of cultured avian embryonic cells by pathogenic microorganisms (e.g. *Mycoplasma* ssp., viruses or bacteria) is more likely to impact the growth of the cells and thereby the yield of harvested cells than to affect human health (Weiskirchen et al. 2023). The risk of microbial contamination during the production process occurs when handling and adding media components to either the cultured cells during the expansion phase, or during harvest or post-harvest handling (Frommer et al. 1993).

As the cell line used by Vow is an immortalised embryonic fibroblast, only pathogenic organisms that can be vertically transmitted from the layer hen to the egg are of concern from a public health perspective. Appropriate testing of cell lines in the early stages of the development of the csMCB is a critical step for reducing potential risk from foodborne microbiological pathogens originating directly from the cultured cells.

In general, avian viruses are low risk for causing human illness, particularly through a food route, due to (1) inability to be vertically transmitted, (2) the attachment specificity of the infecting virus to the host, or (3) the transmission route required to cause illness in humans is mainly through inhalation of viral particles (O'Brien et al. 2021; Bushman et al. 2019).

Mycoplasma gallisepticum and *Mycoplasma synoviae* are the most important pathogenic *Mycoplasma* species found in domesticated birds. They can be transmitted either horizontally (bird-to-bird) or vertically (eggs to chicks), however they are non-pathogenic to humans (OIE 2021).

Vow provided evidence from the cell line supplier characterising cell banks that are GMP compliant and tested according to international guidelines for use in vaccine development (US FDA cGMP – 21 CFR 2022; European Pharmacopeia 2008; ICH 2022). FSANZ reviewed information that supports this claim. Evidence included testing for relevant avian viruses and *Mycoplasma* spp. in the csMCB cells. Sterility testing by Vow was done according to the European Pharmacopeia methods. All results were negative. As there are no reported avian zoonoses transmitted via an oral/food route, microbiological testing of the cell line did not need to include specific pathogens or pathogenic agents associated with foodborne illness. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines recommends extensive screening for viral contamination be performed on the MCB. No virus testing results were reported for the csMCB, but were reported for vWCB.

Antibiotics were only used during the initial cell culture establishment stage of the quail embryo cells by the cell line supplier. Antibiotics are only used for the first two passages of the primary cell culture following isolation. The cells were then cultured for more than 12 months by the cell line supplier in the absence of antibiotics before being sourced by Vow. Vow have undertaken antimicrobial testing of the harvested cells to demonstrate lack of residues.

Upon receipt of the csWCB, Vow established both master and working cell banks to use for cultured quail cell production (expansion).To establish the vMCB and vWCB, cryopreserved csWCB quail fibroblast cell aliquots are thawed and grown to specific cell densities before being aliquoted into units of suitable size to be frozen as the vMCB. Each of these units can be further expanded as required to form the vWCB.

No animal-derived components or antibiotics are utilised by Vow during vMCB and vWCB cell line establishment nor during cell expansion. Negative *Mycoplasma* tests using MycoAlert and PCR results were reported for cell samples when the cell banks were established by Vow, minimising the likelihood that *Mycoplasma* were present.

Appropriate testing of cell lines is a critical step for reducing potential risk from microbiological hazards originating from cell lines or microorganisms from the environment, personnel or media. As part of the initial microbiological testing regime of cells, Vow tested the vMCB cells for species-specific viruses Influenza Type A and Newcastle Disease, endogenous retrovirus, and *Chlamydophila* spp.. Samples of the vWCB cells were tested for *Mycoplasma* spp. All assays returned negative results.

Conclusions

The risk of contamination by foodborne pathogens directly associated with the embryonic fibroblast cell isolation or the quail eggs is very low. There is a low risk of microbial contamination of cells during preparation of the cell banks, as these processes follow established GCCP, and cells undergo extensive screening for known avian pathogens including viruses and *Mycoplasma*. Given the significant differences in production of cultured quail cells versus quail or their meat (i.e. unlikely to be exposed to faecal contamination), known microbiological hazards associated with quail birds have limited relevance to the production of cultured quail cells, other than those vertically transmitted via the germ line.

Notably, any microbiological contaminant introduced during this phase would likely severely impact the growth and yield of the cells, limiting their further use for expansion during production from this point forward.

2.2.2 Cell line stability

Cell line stability has been identified in the literature and in interviews/workshops with government scientists and regulators around the world as a factor to consider in the hazard and risk assessment of cell-cultured food (FAO/WHO 2023; Ong et al. 2023). Transitioning cells from an embryo with cell-to-cell adhesion and signalling, to an immortalised cell line in suspension culture, which then undergoes multiple rounds of cell division, can exert considerable pressure on the genome (FAO/WHO 2023; Franzen et al. 2021). This type of process has been associated with genome instability (e.g. large chromosomal rearrangements) and genetic variation (e.g. accumulation of mutations over time) of cell lines, which may potentially contribute to phenotypic variation in cell-cultured food (Ong et al. 2021; Soice and Johnston 2021).

The purpose of the cell line hazard assessment is to determine if there is any significant genetic instability and, if so, the potential for this to impact food safety. This section examines the results of a whole genome sequencing (WGS) analysis which addresses the overall genetic stability of the cell line. The potential for ectopic expression of egg allergens is examined in Section 2.2.2.2 Phenotypic stability and Section 2.2.3 Potential for allergenicity.

2.2.2.1 Genetic stability

WGS analysis of cultured quail cells

An experimental pipeline was established to compare the genetic signature of quail embryonic fibroblasts following their adaptation to culture and when they are harvested from bioreactors at the end of the production process. Specifically, WGS was performed on genomic DNA extracted from conventional quail (*C. japonica*), vWCB and harvested cells (vCQ). The WGS analysis focused on the identification of genomic variants (single nucleotide variants), and whether identified variants revealed minimal biologically relevant impacts via Gene Ontology (GO) analysis (The Gene Ontology Consortium 2017).

The analysis showed the greatest number of genomic variants occurred when the quail cells adapted to culture conditions during the immortalisation process. These variants remain stable up to the harvest stage, with only a very small proportion of new variants occurring during the production process. GO analysis revealed that the observed genomic variants do not alter biological components or processes that would be indicative of genetic instability.

Vow provided additional WGS studies examining genomic variants following a longer culture period (extra culture cycles) or changing GF combinations in the culture media (see Section 3.2). Under either condition, the observed concordance in genomic variants between vWCB and vCQ were highly similar. GO analysis results were unremarkable and do not alter the conclusion of the study above. Based on these additional studies and within Vow's current scale of production, it can be concluded that extra culture cycles or changing GF combinations in the culture media, does not lead to a substantial increase in the incidence of genetic variations when compared to the large number of variations that occurred during immortalisation.

Conclusions

The results of the WGS analysis provide evidence of the genetic stability of the cell line within Vow's current scale of production and maximum number of culture cycles. Most of the genomic variation occurred during the immortalisation process. This suggests that between different batches of cultured quail cells, and when vWCB is used to seed a new production run, any additional genetic variation is expected to be minor. Overall, the observed genetic variations are consistent with what would be expected for cells in culture and do not themselves raise any specific food safety concerns.

2.2.2.2 Phenotypic stability

Vow measures and monitors their cell line at multiple stages of the production process. FSANZ has examined information from Vow relating to the use of GCCP during production. Full details cannot be provided due to CCI. However, Vow has specified that at the end of the production process the harvested cells are composed of only one cell type: fibroblasts. The cultured quail cells therefore maintain their phenotypic identity from the immortalisation stage through to harvest.

In terms of protein expression, embryonic fibroblasts in culture would be expected to display an altered expression profile compared to differentiated cells in quail meat. As it would be impractical to identify every possible expressed protein in cultured embryonic fibroblasts, a targeted approach was used that focussed only on those proteins that are relevant to the safety of the food or that may have an impact on the whole diet.

Only a small number of dietary proteins have the potential to impair health, because of anti-nutrient properties or by eliciting an allergic reaction in some consumers (Delaney et al. 2008). For cell-cultured food, identifying proteins of interest would be dependent on the animal origin of the specific cell line (see Section 2.2.3).

2.2.3 Allergenic and toxicological hazards associated with quail

Quail embryonic fibroblasts grown in culture do not have a history of human consumption in Australia or New Zealand. Insights into some of the potential hazards associated with consuming cultured quail cells can however be gained from considering the safety of quail meat and eggs, in particular any potential toxigenic or allergenic factors that would also be relevant to fibroblasts derived from a quail embryo.

2.2.3.1 Potential for toxicity

Literature searches did not identify evidence that consumption of quail meat from *C. japonica* is associated with adverse human health effects. Rare cases of adverse health effects (referred to as 'coturnism') have been associated with consumption of hunted European migratory quail (*Coturnix coturnix coturnix*). Toxicity is only associated with certain migratory seasons and flight directions (Yeung et al. 2022; Korkmaz et al. 2011; Lewis et al. 1987; Bellomo et al. 2011). Although the causative agent has not been definitively established to date, seed consumed by the quail is suspected as a source of toxins that then accumulate in the meat (Yeung et al. 2022). Coturnism is therefore not considered to be a relevant hazard for cultured *C. japonica* quail cells.

2.2.3.2 Potential for allergenicity

Quail meat

Quail meat, and poultry meat in general, are not considered priority food allergens of public health concern requiring mandatory food labelling.

Poultry meat allergy is generally considered to be rare. Published data mainly refer to single case reports or small case series so the prevalence of clinical poultry meat allergy is uncertain (Klug et al. 2020; Wanniang et al. 2022). Chicken meat is reported to be the main cause of poultry allergy with many individuals also experiencing symptoms following turkey meat consumption (Wanniang et al. 2022; Hemmer et al. 2016).

A literature search by FSANZ identified reports of only two individuals who self-reported food allergy to quail meat, although allergy was not confirmed by oral food challenge in either case (Kelso 1999; Escribano 1998). Individuals reporting allergy after ingestion of various avian meats have shown positive skin prick test responses to other poultry meats including quail, but the clinical significance of this cross-reactivity has not been confirmed (Kelso 1999; Escribano 1998; González-Mancebo et al. 2011; Wanniang et al. 2022).

It has been suggested that, at least in some cases, allergic reactions to poultry meat other than chicken may be milder or these foods may be well-tolerated (Wanniang et al. 2022; Hemmer et al. 2016). For example, some patients allergic to chicken meat tolerate turkey and other avian meats (Vila et al. 1998). Given this, a decision on avoidance of all other avian meat, including quail, from the diet of an individual with chicken meat allergy should be based on an individual clinical evaluation (Wanniang et al. 2022).

Cross-reactivity between poultry meat and fish has also been reported in some atopic individuals, suggesting fish-allergic and chicken meat-allergic individuals may be at risk of developing food allergy to chicken or fish, respectively (Kuehn et al. 2016; Barbarroja-Escudero et al. 2019). The prevalence of this cross-reactivity appears to be very rare (Kuehn et al. 2016; Barbarroja-Escudero et al. 2019; Hemmer et al. 2016; González-de-Olano et al. 2012; EFSA 2014c), and no cases of cross-reactivity between fish and quail have been reported to date.

Quail eggs

Hen's egg allergy is one of the most frequent causes of food allergy, although it is frequently outgrown in later life (EFSA 2014). Clinical and serological cross-reactivity between hen's egg proteins and those of other bird eggs including quail has been reported (Langeland 1983; Alessandri et al. 2005; Lee et al. 2021; Mitomori et al. 2022). As a result, individuals with hen's egg allergy are generally advised to avoid consuming all poultry eggs, including quail (Lee et al., 2021; Mitomori et al., 2022; The Royal Children's Hospital Melbourne 2016).

Proteins associated with potential cross-reactivity include ovotransferrin, ovalbumin, ovomucoid and lysozyme, all considered major hen's egg allergens (Takahasi et al. 1999; Lee et al. 2021). Lee et al. (2021) reported that the antigenicity of ovomucoid from quail egg white and hen's egg white remained stable during boiling, antigenicity of ovalbumin was weakened and ovotransferrin and lysozyme were not detectable after 15 minutes of boiling.

Quail egg allergy has also been reported in individuals who tolerate hen's eggs, although the incidence appears to be rare. Three case reports and one case series of 5 patients with IgE-mediated quail egg allergy in individuals tolerant to hen's eggs were identified in the literature (Caro Contreras 2008; Micozzi et al. 2016; Ferreira et al. 2020; Ghobadi Dana et

al. 2020). In the case series and one of the case reports, the individuals reported they tolerated quail meat (Micozzi et al. 2016; Ferreira et al. 2020). Three case reports of individuals with food protein-induced enterocolitis syndrome (FPIES), a non-IgE-mediated food hypersensitivity reaction characterised by profuse, repetitive vomiting and diarrhoea, in response to consumption of quail egg were also identified (Sanlidag et al. 2016; Akashi and Sato 2017; Kajita et al. 2019). In all three cases the individuals tolerated hen's egg.

Embryonic fibroblasts contain the complete quail genome, including the genes for egg allergens. However, it is highly unlikely that these genes would be expressed ectopically in embryonic fibroblasts, which maintain their identity in culture. These allergens are expressed by specific cells of mature female quails and in response to specific hormones (Stadnicka et al. 2018). Regardless, allergen residue analysis of the cultured quail cells also tested for the potential presence of egg allergen (Section 2.3.3.4).

2.2.3.3 Additional supporting information – sequence homology

Vow conducted a bioinformatics analysis of similarity of amino acid sequences from *C. japonica* to those of known food allergens. The publicly annotated *C. japonica* genome[3] was used as a basis for assessment of potential allergens in the cultured quail cells. This additional analysis is of limited value for risk assessment purposes because it is entirely theoretical and provides no information that any of the open reading frames (ORFs) identified as encoding putative allergens are actually expressed in embryonic fibroblasts. The results however are briefly summarised below.

An initial screen of all *C. Japonica* ORFs was performed against the AllergenOnline database (v21), searching for full-length alignments by FASTA with identity matches > 50% indicating possible cross-reactivity. ORFs with potential matches were then evaluated using a sliding window of 80 amino acids to find sequences with identities > 35% and an E score of <1e-7[4]. Sequences meeting these criteria were further evaluated for potential allergenicity.

Sequences with a degree of homology to several allergens from chicken meat, chicken eggs and fish were identified. These findings are consistent with reports of potential cross-reactivity between chicken, other poultry (including quail) and fish, and between hen's egg and other bird's eggs. As would be expected, no sequences with similarity to allergenic proteins from the common allergens peanuts, tree nuts, soybean, lupin, milk, sesame, molluscs, crustaceans and cereals containing gluten were identified.

2.2.3.4 Allergen residue analysis

Enzyme-linked immunosorbent assay (ELISA) analyses were performed on Vow's cultured quail cells to assess the presence of gluten, and relevant allergenic proteins from milk (β-lactoglobulin, casein and total milk allergens), egg (ovomucoid), peanut, lupin, sesame seed, mustard seed, fish, crustacean (tropomyosin), mollusc (tropomyosin), almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pistachio, and walnut. Testing was performed on cells produced using the two GF combinations in the culture media used during production: GF 1/GF 2a, and GF 1/ GF 2b (see Section 3.2). None of these allergens were detected. Although they are not allergens, sulphites were also analysed and found to be below the limit of detection.

[3] GenBank/RefSeq GCA_001577835.2

[4] An E score, or expectation score, is a threshold for determining the significance level of sequence similarity in bioinformatic analysis. E scores larger than 1e-7 are not likely to identify matches that would indicate the proteins share immunological or allergenic cross reactivity (Allergenonline.org).

To further support the conclusion that ectopic expression of egg allergens is highly unlikely, Vow has provided the results of egg allergen ELISA testing across multiple batches of cultured quail cells produced with both combinations of GFs (see Section 3.2). In all batches, the results were below the limit of detection. The results support the conclusion that within Vow's current scale of production the ectopic expression of allergens in cultured quail cells is highly unlikely.

Conclusions

Quail meat has a history of safe use without evidence of adverse toxicological effects. Rare cases of illness associated with consumption of hunted European migratory quail are not a relevant hazard for cultured quail cells.

Allergy to quail meat appears to be extremely rare, and neither quail meat or poultry meat in general are classed as major food allergens.

Individuals with hen's egg allergy are generally advised to avoid eggs from other birds, including quail. Quail egg allergy has also been reported in people tolerant to hen's egg, although cases are very rare. However, data provided by Vow supports the conclusion that the embryonic fibroblasts are genotypically and phenotypically stable and do not express egg allergens. Allergen residue analysis of multiple batches of cultured quail cells found the major egg allergen ovomucoid was below the limit of detection.

Taken together, the available information indicates Vow's cultured quail cells are unlikely to pose a food allergenicity concern for the general population. However, as a precaution individuals who avoid quail meat may also wish to avoid cultured quail cells unless tolerance has been confirmed.

2.3 Cell line conclusions

Data provided by Vow confirmed the species of the cell line is *C. japonica*. The risk of microbiological contamination by foodborne pathogens directly associated with embryonic fibroblast isolation or the quail eggs is very low. Evidence assessed confirm the cell line, designated 221523-Fib-Quail, is genetically stable and harvested cells are unlikely to pose a food allergenicity concern for the general population.

3 Method of production

To produce cultured cells for food use, the cell line is expanded to a desired cell density and then harvested. During cell expansion, cells undergo a series of proliferation phases, which both increases the production volume and cell concentration. Cell expansion occurs in a controlled system involving various steps, including culture media and inputs, and multiple culture vesicles (Figure 2). Following cell expansion the cultured cells are harvested using different harvesting techniques (Figure 2).

Production of the cell biomass is managed through Vow's food safety system which includes a HACCP-based food safety plan supported by GHP, cGMP and GCCP. Their food safety system covers processing conditions, cleaning (including CIP), sanitisation and maintenance of premises and equipment. Vow confirmed all materials used in the production of Vow cultured quail cells as meeting the requirements for food grade or pharmaceutical grade ingredients of a purity and quality suitable for their intended use in food.

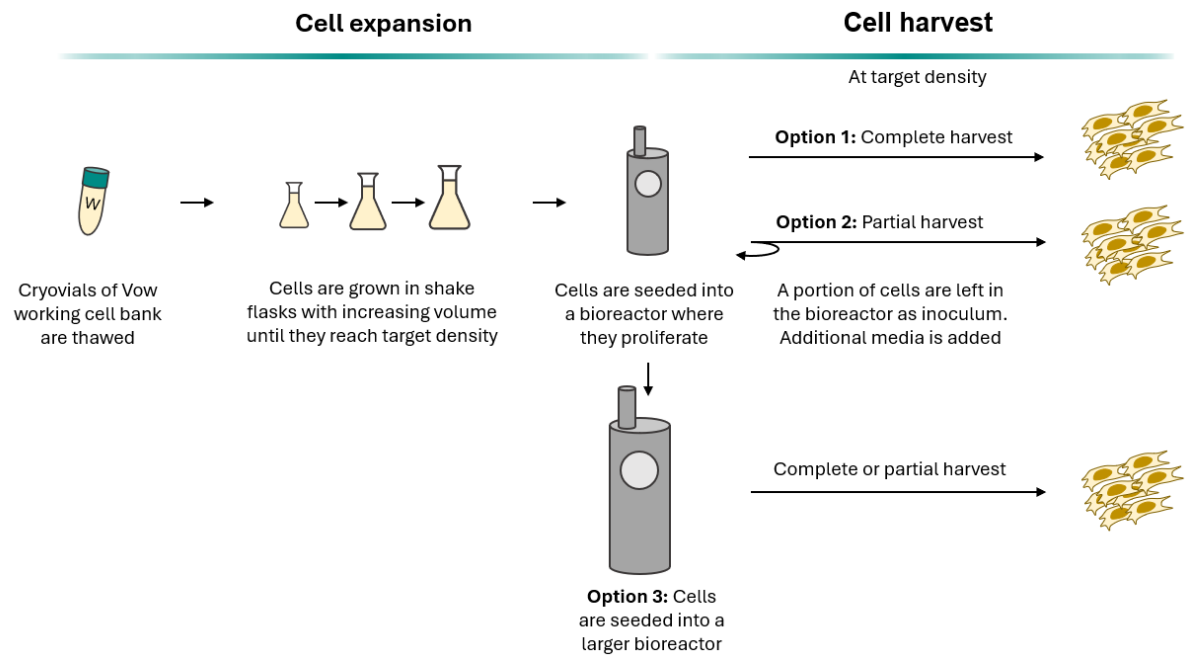

Figure 2 *A schematic overview of the method of production of cultured quail cells*

3.1 Basal media and inputs

Substances used in the production of cultured quail cells include basal media, media additives, growth factors, cryoprotectant and cleaning agents. Collectively, the basal media and other substances / inputs are referred to in this report as culture media. The identity of the individual media components and other inputs is CCI and cannot be disclosed. Full details have been provided to FSANZ for the purpose of this hazard and risk assessment.

FSANZ has assessed the potential health risks associated with exposure to these substances from consumption of cultured quail cells following the approaches set out below:

- substances listed in the Code (e.g. permitted food additives, processing aids, permitted forms of vitamins and minerals, microbial nutrients or permitted for addition to special purpose foods[5]), in particular those permitted for addition to food at levels consistent with GMP, were generally considered to not be of concern, with estimated levels of exposure also taken into account.

- some substances such as certain vitamins, minerals and amino acids were evaluated in the nutrition risk assessment.

- for other inputs, consideration was given to the identity, source, and any hazards associated with these substances.

- the review considered toxicological information and/or risk assessments of the substances by overseas agencies, as well as information on exposure to these substances from conventional quail or other dietary sources.

- exposures to the inputs were estimated based on analytical measurements of the substance in cultured quail cells, or by assuming the entire amount of a particular

[5] While some of these substances are permitted in the Code for specific technological purposes in food, FSANZ is of the view these substances are being used to support the growth of the cells during culture and are not performing a technical function either during food processing, or in the final food.

component in the growth media would be taken up by the cells.

- exposure estimates assumed that 300 g of cultured quail cells would be consumed every day, which is considered likely to be an overestimate.

- estimated exposures were compared to dietary exposures from other sources, endogenous exposures, health-based guidance values (HBGVs) or no observed adverse effect levels (NOAELs) from toxicological studies in laboratory animals.

- when estimated exposures were compared with NOAELs, a margin of exposure (MOE) was calculated.

- For substances which are not genotoxic and carcinogenic, an MOE > 100 is generally considered to indicate a low health concern. No substances that are genotoxic or carcinogenic are used in the production of cell-cultured quail.

- In cases where an MOE < 100 were identified using this conservative approach, Vow measured residue levels of the substance in the cell-cultured quail in order to obtain a more realistic assessment of exposure and refine the risk assessment.

- Potential allergenicity of the media inputs was also evaluated.

3.1.1 Basal media

The quail cells are cultured in a basal culture media provided by a third party, combined with other common cell culture media components such as amino acids, sugars and salts. Many of the added components have a long history of safe use in food. The media components support the growth of rapidly dividing cells, provide fuel for the cells, and help to maintain pH.

The media components were evaluated by FSANZ according to the approach outlined above. No safety concerns arising from the presence of these substances in cultured quail cells were identified.

3.1.2 Media additives

Several media additives are used by Vow to support cell viability and mimic a natural physiological environment. These include additional amino acids and polyamine compounds. For these substances the MOEs are greater than 100, are present at similar or higher levels in conventional quail or other dietary sources and/or are found in the human body. On this basis there are no safety concerns from dietary exposure to these substances from cultured quail cells.

3.1.3 Growth factors

Growth factors (GFs) are a class of polypeptides or proteins that play important roles in the regulation of cell division and tissue growth in an organism[6]. They often have identical or similar amino acid sequences across species and are used in cell culture to promote the proliferation of cells.

[6] There are differences between growth factors, hormones, cytokines and chemokines. For example, hormones are produced by endocrine glands and unlike growth factors they could be non-protein substances such as steroids.

Two recombinant GFs are added to the basal media to support the proliferation of the quail cells. The first GF (GF 1) is porcine and produced in barley seed. The second GF is bovine/porcine[7] and produced in either barley seed (GF 2a) or *Escherichia coli* BL21 (GF 2b). The hazard assessment considered the potential for adverse health effects from allergenicity, toxicity or bioactivity of these substances.

Protein purification of the barley seed results in mixtures that are 20-30% GF with the remainder as barley seed proteins. This mixture is added to the basal media, which means that barley proteins may also be present in the cell biomass. Vow has provided ELISA analysis data to demonstrate that gluten levels are below the limit of detection in the cell biomass (5 – 10 ppm).

Levels of GF 1 (porcine) were measured in uncooked and cooked cultured quail cells using three different approaches (ELISA, Jess Simple Western and Western blot analysis). GF 1 is naturally present in several animal food products, and human forms of the GF are found in human tissues. Levels in one serving of cultured quail cells were within the range of the same type of GF found in one cup of milk, and below levels administered orally in human clinical studies without evidence of adverse effects. In addition, degradation of the GF would be anticipated at cooking temperatures and proteolytic degradation in human gastrointestinal fluids has also been demonstrated (Reference provided (CCI)).

GF 2a (bovine/porcine) was measured by ELISA in uncooked and cooked harvested cultured quail cells, uncooked conventional quail meat and uncooked chicken meat. These measurements found 24.5% of the total amount of GF 2a added during the cell culture process is present in the final uncooked cultured quail cells. Levels in uncooked cultured quail cells are below the levels measured in conventional quail or chicken meat (approximately 60% or 71%, respectively). Limited testing of cooked cultured quail cells suggests cooking further reduces GF 2a content by approximately 80%. Following consumption of cultured quail cells it is expected that GF 2a will be rapidly digested in the gastrointestinal tract. Even if the total amount present in cooked cultured quail cells were to be absorbed intact, this would constitute only a small proportion of the total amount of this GF produced daily in adult humans.

GF 2b (bovine) was measured by ELISA in uncooked and cooked cultured quail cells, conventional quail and beef. Testing showed that < 1.5% of the total amount of GF 2b added is present in the final uncooked product, and below the levels measured in conventional quail and beef. Levels in cooked cultured quail cells were below the lowest level of quantification. Consumption of the levels detected in uncooked cultured quail would be equivalent to consuming only a small fraction of the total amount produced endogenously in adult humans.

Taking these factors into account, use of the recombinant GFs in the production of cultured quail cells at the proposed levels is not expected to pose a safety concern.

3.1.4 Cryoprotectant

A cryoprotectant is used in the cell banking process. Given the very large quantities of medium used during the cell culture and expansion procedures, the cryoprotectant will be substantially diluted and present in the harvested cells at an extremely low level and is not expected to be a safety concern.

[7] This GF is highly conserved between bovine and porcine species.

3.1.5 Antifoam agent

An antifoaming agent is used to regulate headspace foaming in the cell culture bioreactors. The substance used is listed in the Code as a food additive in Schedule 16 of the Code and as a processing aid in Schedule 18. Following the risk assessment approach outlined above, there are no safety concerns from exposure to this substance from consumption of cultured quail cells.

3.1.6 Cleaning agents

Cleaning agents are used to clean the equipment used in the production process. All of the substances used are of low toxicity and intended for use to clean food-processing equipment.

3.1.7 Conclusions

Based on the information provided in this application, no safety concerns arising from the presence of the basal media and inputs in the harvested cells were identified.

3.2 Culture media formulations

During the production process, the cells are grown in one of two culture media formulations (Table 2). The first formulation (culture media 1) contains GF 1 and GF 2a. The second formulation (culture media 2) contains GF 1 and GF 2b.

Vow has provided information and data corresponding to cells grown in either of these two formulations. In the relevant sections, this report specifies which data sets relate to which GF combination.

Table 2 *GF combinations in the culture media*

	GF 1	GF 2a	GF 2b
Culture media 1	X	X	
Culture media 2	X		X

'X' highlights which GF is used in culture media 1 or 2

3.3 Culture expansion

To reach the concentration of cells required for harvesting, the volume of media is increased in a stepwise fashion via a series of expansion and bioreactor seeding steps. At each of these steps there is a risk of microbial contamination occurring (FAO/WHO 2023). A summary of the microbiological hazard identification and description, including expected mitigation and information Vow provided is in Appendix A4.2.

In a scientific literature search, there were no specific studies on microbiological contamination of quail cell lines by bacteria, yeast or fungi. In general, contamination of cell lines by bacteria, yeast or fungi, can occur particularly during passaging or culture splitting steps (Cobo et al. 2007; Frommer et al. 1993; Geraghty et al. 2014; Weiskirchen et al. 2023). Conditions used to grow eukaryotic cells are conducive to growth of mesophilic bacteria, yeast, and fungi. Due to their faster growth rate, these microorganisms are likely to out compete eukaryotic cells during culturing, making contamination easy to observe during expansion phases.

Bacterial or fungal contamination can be detected by changes in pH, turbidity, CO_2 concentration, or by using standard light microscopy checks during cell cultivation (Pauwels et al. 2007). Although viruses and *Mycoplasma* are not detectable by standard microscopy, they may impact on the cytopathology of the cells (Pauwels et al. 2007). Standard compendial sterility tests applied to cell lines can provide an indication of the effectiveness of aseptic processing in preventing general bacterial or fungal contamination. However they are not capable of isolating all potential bacterial and fungal contaminants (WHO 2013).

The data presented in the application including microbial specifications are based on the current scale of production. Vow must ensure when producing at larger scales of production, the monitoring program is revised appropriate to the scale of production, with reference to indicators of process control as identified in Section 4.4.1. Further, testing for potential pathogens such as *L. monocytogenes* should be introduced on product and environment given unknown and unassessed further processing of the cell biomass.

3.3.1 Sources of microbial contamination

There is potential for microbiological contamination of the cell culture during the expansion phase from both inputs (e.g. media and equipment), and handling (e.g. personnel and equipment). The specific steps are thawing cryovials and transferring cells into shake flasks, passaging cells into shake flasks and transferring into sealed stir tank reactors (FAO/WHO 2023; FSA 2023; Hadi and Brightwell 2021). Raw materials and media ingredients may contain bacteria, fungi, and viruses that can contaminate cell cultures. Vow confirmed these inputs are sourced from suppliers of pharmaceutical or food grade reagents which lowers the risk of microbiological contamination. Vow implemented routine process controls verifying the absence of contamination in every batch. Inputs into the expansion media are sterilised prior to addition to the bioreactors. As Vow use non-animal derived media components, the likelihood of microbiological contamination is further reduced.

There is no data on microbiological contamination rates at different points of production of cultured quail cells or animal cell-cultured products for use as either a food or food ingredient. There are no published challenge studies for quail embryonic fibroblast cells describing cell culture parameter changes and potential for growth of foodborne pathogens (e.g. bacteria, fungi, yeasts, moulds, or viruses) if contamination occurred and was not detected before entering sealed bioreactors.

Since mesophilic bacteria, yeast or fungi contamination is likely to be visually observable in the shake flasks, disposal before entering sealed bioreactors is also likely. The cell biomass in a bioreactor can be considered to be “microbiologically sterile” (FSA 2023). While these are reasonable assumptions, challenge studies with surrogates for foodborne pathogens would provide more certainty and data to inform risk analysis of these production systems in future.

Non-cytopathic viruses and *Mycoplasma* spp. infections are more likely to proceed unnoticed in the cell line, coming from either the original cells, personnel, media or the environment. Visual inspection of the cell biomass will not be sufficient to detect *Mycoplasma* or viral infections that do not affect measurable culture parameters. Prevention of infections at cell expansion stage will be reliant on the use of well characterised cell lines and consistent and robust aseptic processes.

3.3.2 Control of microorganisms

Biotechnology products produced in similar bioreactor settings typically undergo purification to generate the final product which removes the production organism and manages microbial hazards. However, eukaryotic cells cultured for food do not include steps for microorganism

control or cell removal during production. Currently, there are no methods available for inactivating viruses during culturing of cells for food uses. Methods for detecting viruses if present in avian cells are limited. Using guidance for evaluating safe production of biotechnology products from cell lines of human or animal origin is relevant for minimising risks in cell lines used for food production (EMEA 1997). In line with this guidance, cell lines destined for food production should be free from microorganisms that can cause foodborne illness.

3.3.3 Cleaning and sanitising

Equipment and surface cleaning compounds include a range of washing, disinfectants and sanitisers that can be used on food and non-food contact surfaces to remove any biological material build up (e.g. biofilm) that might promote the growth of or harbour bacteria. Noting that some of the sanitisers used in CIP might be no rinse, ATP swabs are used to monitor the efficacy of sanitising procedures, particularly on food contact surfaces prior to use.

3.3.4 Management of cell culture process

Vow provided a GCCP plan with reference to internal production procedures. Management strategies applied to reduce risk from potential microbiological hazards consistent with GCCP, were in line with published approaches (Bal-Price and Coecke 2011; OECD 2018; Pamies et al. 2022). Vow's HACCP plan included procedures for monitoring the cell culture expansion phase for bacterial, fungal and yeast contamination and for managing cell culture batches that did not meet their microbial specifications (Table 5).

3.3.5 Conclusions

As there is no specific step within the production process that will reduce or eliminate microbiological contaminants, the most critical controls will be at steps where microbiological hazards can be prevented from entering or increasing during the process. This requires using a systematic approach to the management of the potential for:

(1) contamination of the acquired cells from source animal, reagents, or environment;
(2) contamination from manual handling;
(3) contamination from any inputs during production;
(4) facility environmental contamination; and
(5) inadequate cleaning and sterilisation of equipment.

3.4 Method of production conclusions

Evaluation of the basal media, media additives, growth factors, antifoam, cryoprotectant and cleaning agents used during the production process indicates there are no safety concerns from exposure to these substances from consumption of Vow harvested cultured quail cells. Barley proteins may be present in the harvested cells, but gluten levels are below the limit of detection.

Adherence to a HACCP-based food safety system underpinned by good practices that accurately identified critical controls is important in reducing the microbiological risk for cell-cultured food production.

4 Harvested cells

This section covers harvesting of cells and the harvested cell biomass. Further processing of cells post-harvest and processing the final food is not part of this application and was not considered in the microbiological hazard identification (Figure 3). FSANZ notes further processing of the cell biomass is food handling under Chapter 3 of the Code and the food business will need to comply with relevant food safety standards.

The microbiological, nutrition and dietary exposure assessments and specifications below apply to the harvested cells, not the final mixed food (Figure 3).

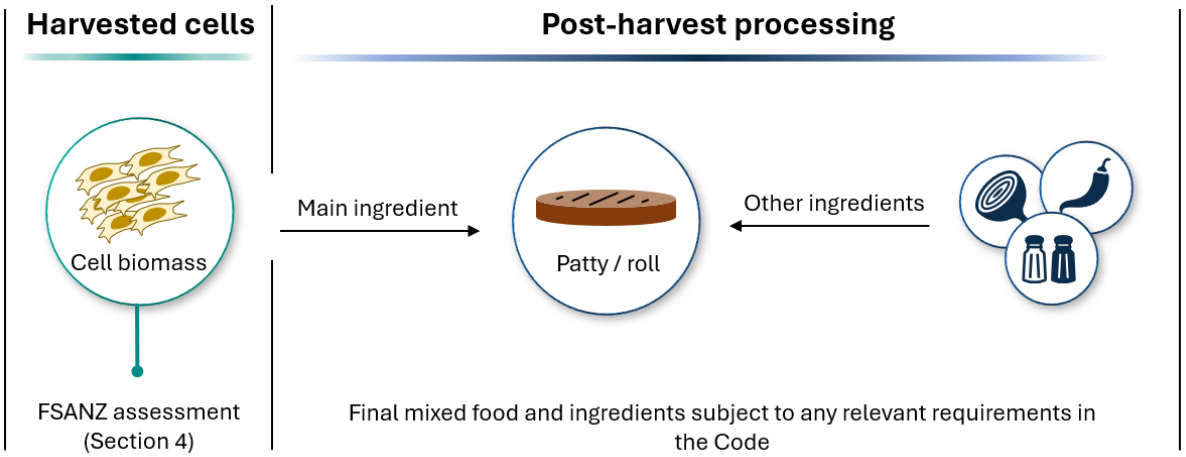

Figure 3 *Schematic representation of the scope of this section*

4.1 Microbiological hazard assessment

4.1.1 Harvesting process

Once the cell line has been expanded to the desired cell density, the cells are harvested and concentrated via centrifugation, collected, packaged and frozen. The cells will be thawed, additional ingredients added and then re-frozen. Frozen harvested cells are further processed into final food products. FAO/WHO (2023) guidance recommends harvesting cells to maintain cell/tissue integrity and avoid microbial contamination. A summary of the microbiological hazard identification and description, mitigation options and information provided by Vow is in Appendix A4.3.

Vow provided information the cells harvested from the bioreactor will be cooled within a timeframe and to a temperature that doesn't support microbial growth (i.e. hard frozen). The time taken to cool harvested cells for their current scale of production was validated but not microbiologically validated. The risk will be dependent on adherence to good practices to maintain the cell biomass's aseptic status.

4.1.2 Harvested cells

During this stage, potential microbiological hazards from ingredients, personnel, equipment, and the environment may contaminate the product. The harvested cells are a concentrated, uniform and homogenous cell biomass; any microorganisms present are likely to be evenly distributed throughout the product during harvesting and mixing in additional ingredients.

This is in contrast to conventional comminuted or minced/ground meat products where microorganism distribution is likely to be more heterogenous and, at least initially, limited to the meat surface which is internalised into the product during further processing (i.e. uneven distribution). The cell biomass is not equivalent in structure or thus risk to that of comminuted meat products. It would require a thorough cook step to ensure microorganisms if present throughout the product are adequately mitigated (i.e. 6D reduction). As the harvested frozen cultured quail cells are the main ingredients in a final mixed food, uniform spread of hazards should be factored into additional processing, including the final shaping and packaging (Appendix A4.3 and A4.4).

The cultured cells whilst in the bioreactor are considered to be 'sterile'. There should be no associated microflora (including potential foodborne pathogens). The greatest contamination risk occurs once they are exposed to the environment, including food contact surfaces and other potential sources of contamination. Nutrient and water availability as well as the neutral pH of the residual growth medium will support growth of most bacterial species if present in the cell biomass post-harvest. Furthermore, the cell biomass will act as a cryoprotectant during freezing, protecting microorganisms (both bacteria and viruses) within the frozen product. Temperature control of the biomass once harvested will be the key limiting factor for most foodborne bacterial pathogens.

Application of GMP/GHP to the production of harvested cells (and final food product) is required as its shelf-life, packaging and storage conditions (e.g. further processing with additional ingredients, directions for use and consumer preparation) will influence the final microbiological safety of the product. There is currently no evidence on the persistence of foodborne pathogens, viral or bacterial, in cultured cell food products. There is also no data reported in literature on microbiological growth potential or stability of cell-cultured food.

Listeria monocytogenes is a recognised food pathogen particularly in ready-to-eat (RTE) foods which can survive within many processing environments (see Section 3.3.1). *Listeria* has been identified as a potential hazard that could be introduced during the harvesting and post-harvest stages of cell biomass production. Given a lack of natural flora associated with the cells in the bioreactor, there is potential for pathogenic organisms such as *Listeria monocytogenes* to proliferate to a higher level during cooling and cold storage compared to traditional meat products.

Microbiological stability analysis of the harvested cell biomass confirmed *Listeria monocytogenes* is able to grow during refrigerated storage; *Listeria monocytogenes* should be considered as a significant microbiological hazard which must be managed during harvesting, packaging, storage and further processing. This is particularly relevant once production is scaled up and the volume of cell biomass increases; it will take longer to chill and then freeze a larger volume of cells.

The final harvested cell biomass is a potentially hazardous food (PHF) as defined in Standard 3.2.2 of the Code; i.e. it supports microbial growth. Identifying it as a PHF will ensure it is kept under temperature control throughout the supply chain to final consumption.

Vow advise harvested cells will be further processed and subject to a cook step before consumption and thus is not a RTE food. While this stage is not part of this application and was not assessed, it is food handling and must comply with Chapter 3 food safety standards. The post-harvest processing stage, including use of other ingredients used to formulate the final mixed foods will need to meet with any relevant requirements in the Code.

4.2 Nutrition risk assessment

4.2.1 Objectives of the nutrition risk assessment

The objectives of the nutrition risk assessment were to:

- compare the composition of the harvested cells to comparison foods
- evaluate whether the consumption of the harvested cells would cause a nutritional imbalance in the diet
- determine the effect of the harvested cells on the absorption of other nutrients.

4.2.2 Approach for the nutrition risk assessment

The nutrient content of harvested cells was compared to conventional quail (lean flesh with skin) using data from the Australian Food Composition Database (FSANZ 2023) and compared to the nutrient reference values (NRVs) including estimated average requirement (EAR), adequate intake (AI), suggested dietary target (SDT) or upper level of intake (UL) where relevant, from the Nutrient Reference Values for Australia and New Zealand (NHMRC and MoH 2006). The NRVs can vary across age groups and sex, due to variations in body size across age groups or changed nutritional requirements during different life stages or pregnancy and lactation. For this assessment, the percentage EAR provided per serving of food is reported for men aged 19-50, and percentage AI is reported for men aged 19 and over. Whist the EAR is usually used to estimate the proportion of a population with inadequate intakes, and the AI gives an indication of median intakes, these NRVs are used for this assessment as a way of indicating the scale of impact that consumption of cultured quail cells could have within the diet.

Vow has provided compositional data for harvested cells grown using two sets of culture media that differ in the GF combinations used. Details of the nutrient composition of harvested cells grown under both conditions are provided in Appendix-I.

Conventional quail is consumed by a small proportion of the population. Less than 1% of respondents aged 2 years and above reported consuming quail in the 2011-2012 Australian National Nutrition and Physical Activity Survey[8]. There was no consumption reported for the New Zealand population in the most recent national surveys. The assessment therefore also included chicken breast (raw lean flesh) as a comparison food (Appendix-I A1.1 – A1.5).

According to Vow, harvested cells are not expected to replace quail or any other food group in the diet to a major extent. They anticipate that a frequent or regular consumer may consume harvested cells once a week as a conservative estimate, and has indicated that a single serving size will be 300 g. For the consumption of conventional quail, Vow stated the 5th percentile weight of meat per quail is 160 g and the 95th percentile is 200 g. For the purposes of this assessment FSANZ used the midpoint of 180 g as the serving size for conventional quail. A serving size of 142 g was used for chicken breast. The estimation of serving sizes is discussed in Section 4.3.5.2 of the Dietary Intake/Exposure Assessment.

[8] Details of the National Nutrition and Physical Activity Survey 2011-2012 are provided in Section 4.3.3 *Food consumption data used and population groups assessed*

4.2.3 Composition of the harvested cells vs comparison food/s and potential effect of consumption on nutrient balance in the diet

4.2.3.1 Macronutrient content

Protein content

Vow provided proximate analysis data of harvested cells grown using two culture media formulations (Table 2), as well as commercially available *Coturnix japonica* from Australia and Singapore (3 birds from each location) and Australian *Colinus* (3 birds) that were raised for consumption. Some differences in nutrient content of harvested cells were observed depending on the culture media used. Amino acid composition data were also provided for harvested cells and *Coturnix japonica* from Australia and Singapore (3 birds from each location; Appendix-I A1.1).

Harvested cells contain an average of 9 g protein[9] (8.8 or 9.5 g depending on the culture media used) per 100 g, conventional quail contains 18.2 ± 1 g protein /100 g (Australian *Coturnix japonica*), 18.8 ± 0.6 g/100 g (Singapore *Coturnix japonica*), 21.2 ± 0.1 g/100 g (Australian *Colinus*). Chicken breast contains 18.5 g protein/100 g. Based on a 300 g serving size for harvested cells and 180 g for conventional quail, this equates to approximately 27 g and 32.8 to 38.2 g of protein per serve of harvested cells and conventional quail respectively.

The EAR for protein is 52 g/day for men aged 19-70, and 37 g/day (0.75 g/kg body weight/ day) for women aged 19-70 (NHMRC and MoH 2006). Data published by the Australian Bureau of Statistics indicate that the majority of Australian respondents aged up to 70 years (99.4 – 99.9%) consume at least the EAR of protein in the diet (Appendix-II A2.1), although 13.7% of Australian males and 3.8% of females over the age of 70 years have inadequate protein intake (below the EAR), due to the increased protein requirement for this age group (ABS 2015c). A similar pattern is observed for New Zealand (Appendix-II A2.2). Harvested cells are expected to be a niche product that will initially be consumed in restaurants. Vow anticipates that a frequent or regular consumer may consume them once a week. Therefore the decreased protein content in harvested cells compared to conventional quail is expected to have a negligible impact on total dietary protein intake.

Amino acid content

Essential amino acids cannot be synthesised in the body and therefore must be obtained from the diet. FSANZ compared the essential amino acid content of harvested cells to conventional quail and chicken meat, light and dark. No data for chicken breast were available. In most cases the essential amino acid content of harvested cells is lower than chicken meat, irrespective of the culture media used. When grown with culture media 2 the essential amino acid content of harvested cells is lower than or similar to conventional quail, and when grown with culture media 1 it is similar to or higher than quail (Appendix-I A1.1). However, EARs have not been set for individual amino acid intakes and as discussed above, the protein intake of the majority of Australians and New Zealanders is sufficient. Also, cell-cultured quail is not expected to be consumed regularly. Therefore the amino acid content of harvested cells is not a nutritional concern.

[9] The protein specification for harvested cells of > 4 g/100 g reflects the alternative quantification method used by Vow, however the validated laboratory analysis indicates a protein content of 9 g/100 g.

Fat content

Harvested cells contain less total and saturated fat, monounsaturated, polyunsaturated and trans fatty acids compared to conventional quail (Appendix-I A1.2). The total fat content of harvested cells is 1.5 and 1.6 g/100 g depending on the culture media used, compared to 11 g/100 g in conventional quail and 0.8 g/100 g in chicken breast (no standard deviation provided). Therefore a single serving contains no more than 4.9 g total fat; a serving of conventional quail contains 19.8 g total fat and chicken breast contains 1.14 g. EARs or AIs have not been set for total fat as it is the type of fat consumed that relates to essentiality and health outcomes (NHMRC and MoH 2006).

The saturated fat content of harvested cells is 0.63 g or 0.67 g/100 g compared to 3.3 g/100 g in conventional quail and 0.25 g/100 g in chicken breast. A single serving would contain no more than 2.0 g saturated fat, compared to 5.9 g in conventional quail and 0.34 g in chicken breast. The Australian Dietary Guidelines (NHMRC 2013) recommend limiting the intake of foods containing saturated fats, and therefore the lower saturated fat content of harvested cells compared to conventional quail would be a nutritional advantage.

Harvested cells contain no more than 40.5 mg/100 g trans fat, conventional quail contains 62.4 mg/100g and chicken breast contains 3.78 mg/100 g. A single serving of harvested cells contain up to 122 mg trans fat, with a similar quantity provided by a serving of conventional quail (112 mg). A serving of chicken breast contains 5.4 mg trans fat. The World Health Organization Report on Diet, Nutrition and the Prevention of Chronic Disease (WHO 2003) recommends that trans fatty acids contribute no more than 1% of total dietary energy. The estimated energy requirements for Australian adults is between 6100 and 18600 kJ per day, depending on sex, BMI and physical activity level (NHMRC and MoH 2006). Based on the lowest estimate of energy requirements, one percent of dietary energy intake of 6100 kJ would be provided by 1.6 g trans fat. Previous estimates of trans fatty acid intakes by FSANZ (2009a) showed Australians and New Zealanders consume around 0.5% and 0.6% of energy from trans fats respectively from the total diet, derived from mean intakes of 1.3-1.6 g/day and 2.7-3.8 g/day for 95th percentile consumers, with ruminant sources being the major contributors. Therefore a single serving of harvested cells would contain a small proportion of the recommended daily limit of trans fat and would be within normal daily variation of daily intakes from the total diet.

Harvested cells contain less polyunsaturated fatty acids than conventional quail or chicken breast, 10 mg/100 g compared to 2.57 g/100 g and 0.16 g/100 g respectively, equating to 30 mg per serving of harvested cells, 4.6 g per serving of conventional quail and 280 mg per serving of chicken breast. Dietary n-6 and n-3 polyunsaturated fatty acids with chain lengths of 18 or more carbons cannot be synthesized in the body and therefore are essential in the diet. AIs have been determined for several omega-3 and omega-6 fatty acids including linoleic acid (13 g/day), α-linolenic acid (1.3 g/day), long-chain omega 3 fatty acids (EPA, DHA and DPA) (160 mg/day combined) (levels for men aged 19+). Mean usual intakes for Australian adult males are 8-11 g/day for linoleic acid, 1.4 -1.6 g/day for α-linolenic acid and 229-270 mg/day for long-chain omega-3s (ABS 2015c; Appendix-I A1.3).

A serving of conventional quail provides between 24% and 35% of the AIs for those essential fatty acids with an established AI (including linoleic acid, linolenic acid and EPA, DPA and DHA combined) however chicken breast (and most mammalian meats[10]) are poor sources of essential fatty acids (chicken breast: 1-12% AI; Appendix-I A1.3). Vow did not provide data on the essential fatty acid content of harvested cells however the low concentration of polyunsaturated fatty acids (and therefore essential fatty acids) present in

[10] Data in food composition database (FSANZ 2023)

harvested cells are likely to be similar to other meats in the diet which may be replaced by harvested cells. Based on the limited consumption of conventional quail in the Australian and New Zealand diet, FSANZ does not consider that a nutritional disadvantage would occur in terms of a potential decrease in polyunsaturated fat consumption following the addition of harvested cells into the diet.

Moisture content

Proximate analysis indicates that harvested cells have a mean moisture content of 87 or 88 g /100 g depending on the culture media used. Conventional quail contains 69.8 g/100 g moisture and chicken breast contains 75.6 g/100 g.

Carbohydrate content

Data provided by Vow indicates that cultured harvested cells contains up to 1 g/100 g carbohydrate. Conventional quail and chicken breast do not contain carbohydrate. Carbohydrate content was not considered further.

4.2.3.2 Micronutrient content

Vitamins

Vow provided data on the vitamin content of harvested cells including retinol, thiamin, riboflavin, pyridoxine, biotin, folate, niacin, cobalamin, and vitamin C. A detailed assessment of cobalamin (vitamin B_{12}), biotin and folate was undertaken because levels for these vitamins were relatively high in the harvested cells.

Cobalamin (vitamin B_{12})

The cobalamin content of harvested cells is 457 or 619 µg/100 g and 1371 or 1857 µg/300 g serving, depending on the culture media used. This equates to 690 or 929 times the EAR for cobalamin (2 µg/day for men and women aged 19+). Mean usual intakes of cobalamin range from 2.6-5.4 µg/day for children aged 2-18 years and from 3.2-5.8 µg/day for adults aged 19+ in Australian and New Zealand (Appendix-II A2.5, A2.6).

No ULs have been set in Australia and New Zealand for adults or children due a lack of evidence of adverse effects at high doses of cobalamin (NHMRC and MoH 2006). Schedule 17—4 of the Food Standards Code—*Permitted uses of vitamins and minerals* sets limits on the maximum amount of vitamins and minerals permitted in certain foods, for example analogues of meat and extracts of meat, vegetables or yeast. No maximum limit of cobalamin concentration in a food category relevant to harvested cells has been set.

No dosage limits have been set by the Therapeutic Goods Administration for the sale of cobalamin in dietary supplements including cyanocobalamin, mecobalamin and hydroxocobalamin (TGA 2023). Over the counter vitamin supplements in Australia are currently available at a daily dose of up to 1000 µg cobalamin.

Bioavailability of cobalamin

It has been estimated that no more than 1.5–2.5 µg cobalamin can be absorbed per meal through receptor mediated absorption (Heyssel et al. 1966; Scott 1997). In addition, approximately 1% of cobalamin intake is absorbed by diffusion (Chanarin 1979; Carmel 2008).

International position on upper level of intake for cobalamin

The 2015 EFSA Scientific Opinion on dietary reference values for cobalamin reported that no adverse effects have been associated with excess intake from food or supplements in healthy individuals, including from long term oral or parenteral administration of daily doses of 1 to 5 mg given to patients with compromised cobalamin absorption. Cobalamin was not found to be carcinogenic or genotoxic *in vitro* or *in vivo*, and no evidence of teratogenicity or adverse effects on fertility or post-natal development were identified. Based on these findings no tolerable upper intake level was set (EFSA 2015).

The US Food and Nutrition Board did not establish an upper limit for daily cobalamin intake based on its low potential for toxicity (Institute of Medicine 1998).

Health Canada has not set an upper limit for cobalamin due to a lack of suitable data but notes this does not mean there is potential for adverse effects resulting from high intakes (Health Canada 2010).

FSANZ undertook a literature search in Pubmed on 27 April 2023 to identify recent publications (since the publication of Australian and New Zealand NRVs in 2006) relating to any adverse health effects of high doses of cobalamin ingestion[11]. Four relevant studies were identified, including a case study (Morales-Gutierrez et al. 2020), a pilot study (Mallone 2020), a randomised controlled trial (Kaji et al. 2019), and a systematic review (Wang et al. 2018).

Case study

A 24 year old Hispanic woman with multiple autoimmune conditions including pernicious anaemia, psoriasis and Hashimoto's thyroiditis was treated with cyanocobalamin to treat deficiency. The patient received 1 mg cyanocobalamin for six days and then 1 mg per week for two weeks. The treatment was stopped due to discomfort and then restarted with a dose of 1 mg/day for four more days. The patient received a total dose of 12 mg, during which time she developed symptoms that included acne, palpitations, anxiety, akathisia, facial ruddiness, headache, and insomnia. Symptoms improved two weeks after ceasing treatment. The author concluded that symptoms may have been due to high doses of cyanocobalamin (Morales-Gutierrez et al. 2020).

Pilot study

An Italian study by Mallone et al. (2020) investigated the neuroprotective effect of oral high-doses of thiamin (300 mg), pyridoxine (450 mg) and cobalamin (1500 µg) administered daily for 90 consecutive days to 16 patients (mean age 36.3 ± 7.3 SD (standard deviation) years) with stable relapsing-remitting multiple sclerosis. None of the patients had low serum cobalamin when commencing the study. The authors reported that no treatment-related adverse effects were observed in patients during the whole follow-up period.

Randomised controlled trial

A randomised, double-blind placebo controlled study conducted at 51 sites in Japan evaluated the efficacy and safety of intramuscular ultra-high doses of methylcobalamin (25 and 50 mg) or placebo in 370 adult patients with amyotrophic lateral sclerosis (Kaji et al.

[11] Search terms: (((((((high dose) OR (acute dose)) OR (overdose)) OR (excess)) OR (toxicity)) OR (toxic dose))) AND (((((((vitamin b-12) OR (cobalamin)) OR (vitamin b12)) OR (methylcobalamin)) OR (adenosylcobalamin)) OR (hydrocobalamin)) OR (vitamin b 12)) Filter from 2006 – 2023.

2019). Allocated drugs were administered twice a week for 182 weeks. The authors considered that the incidence of treatment-related adverse effects were similar in placebo and treatment groups: 4.1% (5/123), 7.3% (9/124) and 5.7% (7/123) in the placebo, 25 mg and 50 mg groups respectively, with no clinically significant changes in the results of laboratory tests, vital signs or ECG among groups. No further details were provided.

Systematic review

In 2018 a Cochrane systematic review of the evidence for the effect of oral versus intramuscular administration of cobalamin for treating deficiency found that adverse effects from high doses (1000 µg/day) of cobalamin were rare in both groups (Wang et al. 2018). Three parallel randomised controlled trials were used in the body of evidence. These studies included 153 participants (oral group: n=73; intramuscular group: n=79) from three countries, mean ages 39-72 years with megaloblastic anaemia due to cobalamin deficiency. Participants from two of the studies had gastric co-morbidities. Oral doses of 1000 µg/day were used in two studies and a dose of 2000 µg/day was used in one study, with duration ranging from 3 to 4 months. All trials used an intramuscular dose of 1000 µg that was initially administered either daily or every second day, with decreasing frequency over the study period. One trial stated that no treatment-related adverse events were observed in either the oral or intramuscular treatment groups. One trial reported that 2 of 30 participants (6.7%) in the oral cobalamin group left early due to adverse events. One trial did not discuss adverse events. The authors concluded that the quality of evidence was low or very low in the three studies due to risk of performance and detection bias and serious imprecision.

It is noted that the study population are individuals with megaloblastic anaemia and therefore may include individuals with impaired cobalamin absorption. Therefore the findings regarding adverse effects to high doses of cobalamin may not be relevant to a normal healthy population.

Based on the limited bioavailability of cobalamin, the absence of ULs in Australia and New Zealand or internationally, the lack of adverse effects in individuals consuming high doses of cobalamin and expected infrequent consumption, FSANZ does not consider the cobalamin content of harvested cells will be a nutritional concern.

Biotin (vitamin B_7)

Harvested cells contain 86 or 87.3 µg/100 g biotin, depending on the culture media used, compared to 5.6 µg/100 g in conventional quail and 2.1 µg/100 g in chicken breast. A 300 g serving contains no more than 262 µg biotin compared to 11.7 µg per serving of conventional quail and 3 µg per serving of chicken breast. This equates to approximately nine times (860% or 873%) the AI compared to 34% in conventional quail and 10% in chicken breast.

Biotin is produced by intestinal bacteria, which makes dietary requirements uncertain (Wahlqvist 2002). No ULs have been set for biotin intake, which is due to insufficient evidence of adverse effects (NHMRC and MoH 2006).

International positions on upper level of intake for biotin

No reported adverse effects from biotin intake in humans or animals were found by the US Food and Nutrition Board, and insufficient data were available to set ULs (Institute of Medicine 1998). The 2014 EFSA Scientific Opinion on dietary reference values for biotin did not define ULs for biotin intake (EFSA 2014a). Health Canada have not defined ULs for biotin (Health Canada 2010) but state this does not mean that there is no potential for adverse effects resulting from high intake.

FSANZ undertook a literature search in Pubmed on 11 May 2023 to identify any recent studies published since the development of the Australian and New Zealand NRVs, relating to potential adverse effects of high intake of biotin[12]. Six relevant human studies were identified (Sedel et al. 2015; Tourbah et al. 2016; Maillard et al. 2019; Couloume et al. 2020; Cree et al. 2020; Juntas-Morales et al. 2020). Three rodent studies were excluded due to the use of very high doses of biotin (equivalent to 98 mg to 64 g biotin/day in a 65 kg adult) that were not considered relevant to the consumption of harvested cells (Sawamura et al. 2007, 2015; Shiozawa et al. 2022).

A pilot non-controlled, unblinded study from three centres in France was undertaken in 23 patients aged 26-75 years (ratio of M:F not provided) with primary and secondary progressive multiple sclerosis (MS) to determine the clinical efficacy and safety of high doses of biotin (100-300 mg/day for 2 to 36 months; mean duration 9.2 months). No adverse effects were reported in 20 patients and 2 patients experienced transient diarrhoea. One patient died one year after treatment commencement and one patient died 36 months after treatment commencement but the deaths were not considered to be treatment-related (Sedel et al. 2015).

A double-blind placebo controlled French study was undertaken to determine the efficacy and safety of high-dose biotin (300 mg/day; female n= 53; male n=50; mean age 51.8 ± 9.1 (SD) years) or placebo (female n=30; male n=21; mean age 50.7 ± 8.4 years) in patients aged 18-75 with progressive MS for 12 months, which was followed by an additional 12 months for both groups. The author reported that the incidence and distribution of adverse events during the placebo-controlled phase was similar between groups (Tourbah et al. 2016).

A single case study reported a 22 year old female patient with progressive MS who experienced severe transient myopathy following oral doses of 300 mg/day for 12 months (Maillard et al. 2019). Symptoms reversed in the 6 months after treatment was withdrawn. The author noted that symptoms were rare, and were not reported in over 7000 patients with primary MS undergoing similar treatment in France.

In a non-controlled prospective French study 178 patients aged 52 ± 9.4 years (ratio of M:F not provided) with primary or secondary progressive MS were treated with high dose pharmaceutical grade biotin (300 mg/day) for 12 months in order to determine efficacy to improve disability and dexterity (Couloume et al. 2020). Adverse effects were reported in 25 patients and were mostly mild or moderate including asthenia, edema, skin problems and disruption of thyroid assays. However a placebo group was not included in the study for comparison.

High dose pharmaceutical-grade biotin was used in a randomised, double-blind, parallel placebo-controlled trial undertaken at 90 academic and community MS clinics in 13 countries. Patients aged 18-65 years with primary or secondary progressive MS were randomly assigned oral biotin (100 mg 3 times per day; female n=175; male n=151) or placebo (female n=170; male n=146) for 15 months. Treatment-emergent adverse events were similar in both groups with 277 (85%) reported in the treatment group and 264 (84%) in the placebo group. Serious treatment-emergent adverse events were reported in 26% of each group. One person died in the treatment group however this was not considered to be related to the treatment. No deaths occurred in the placebo group (Cree et al. 2020).

[12] Search terms: (((high dose) OR (acute dose) OR (overdose) OR (excess)) AND ((toxicity) OR (toxic) OR (adverse effect) OR (adverse)) AND ((biotin) OR (Vitamin B7))) Filter from 2007 – 2023.

A pilot study of 30 French patients aged 25-80 years with probable or definite amylotrophic lateral sclerosis participated in a single centre randomised, double-blind placebo controlled trial to determine the safety of high dose (300 mg/day for 24 weeks; female n=6; male n=14). The authors reported that the treatment was safe and well tolerated (Juntas-Morales et al. 2020).

Based on the absence of ULs for biotin set by regulatory agencies, recent evidence reporting very rare and mild adverse effects following the long term administration of high daily doses and expected infrequent consumption, FSANZ does not have concerns regarding the level of biotin in a serving of harvested cells.

Folate (vitamin B_9)

Harvested cells contain 217 or 268 µg/100 g total folate[13] and provide 651 or 804 µg /300 g serving. Data from the Australian Food Composition Database (FSANZ 2023) indicates that conventional quail, chicken and duck breast, and turkey breast/hindquarters do not contain natural folate. Vow provided data for total folate but not folic acid. The total folate in harvested cells may be in the form of folic acid that is introduced during the production process.

Natural folate found in food is approximately 50-60% bioavailable; folic acid in fortified foods or supplements is approximately 85% bioavailable (NHMRC and MoH 2006). Natural food folate is considered to be safe, and high intakes are not associated with adverse effects (Butterworth and Tamura 1989; Institute of Medicine 2000). However studies have reported that folic acid has the potential to mask megaloblastic anaemia due to cobalamin deficiency and delay the timely diagnosis and treatment of the disease allowing irreversible combined degeneration of the spinal cord to progress (Institute of Medicine 2000; EFSA 2014b). Therefore the NHMRC set a lowest observed adverse effect level (LOAEL) for folic acid of 5 mg/day (NHMRC and MoH 2006). An uncertainty factor of 5 was used because the available data was not well controlled, the adverse effects were severe and no NOAEL was available. Therefore the UL for folic acid is 1000 µg for men and women aged 19 years and over, and pregnant and lactating women aged 19-50 years. The UL for boys and girls (including pregnant and lactating girls/women) aged 14-18 years is 800 µg/day. The UL for children aged 9-13 years is 600 µg/day, 400 µg/day for children aged 4-8 years and 300 µg/day for children aged 1-3 years (NHMRC and MoH 2006). Mean usual intakes of folic acid for Australians range between 154-278 µg/day (ABS 2015b). No ULs are set for natural folate.

If present as folic acid, a single serving of harvested cells would reach the UL for boys and girls aged 14-18 years, if grown with culture media 1, based on a serving size of 300 g cells (excluding other ingredients used to prepare the final cell-cultured quail product). As a comparator food, the mean consumption for chicken meat in boys and girls in Australia (aged 14-18 years) and New Zealand (aged 15-18 years) is 91 – 159 g per day (Table A3.2). A serving size of 300 g would exceed that of a high consumer (90th percentile) for girls in Australia and New Zealand (186 g and 206 g respectively), and Australian boys (182 g). New Zealand boys may consume a comparable serving size (369 g at the 90th percentile). However this is considered to be an overestimation of daily consumption as the New Zealand dietary intake data is based on only one day of food consumption data, as explained in section 4.3.3. Given cell-cultured quail is expected to be sold as a niche product which is not likely to be widely or frequently consumed, as well as the current comparative serving size of consumers aged 14-18 years, it is unlikely the UL for folic acid from the consumption of cell-cultured quail would be consistently exceeded.

[13] Total folate includes natural folate and folic acid; it is the sum of the two components with no conversion factor to account for potential differences in potency.

As noted, folic acid has the potential to mask megaloblastic anaemia due to cobalamin deficiency, however the usual intake of cobalamin for the majority of the population group in Australia and New Zealand is at or above the EAR (92.1-99.9%; Appendix-II A2.5, A2.6), and a conservative uncertainty factor has been applied in establishing the folic acid UL. In addition, the UL is defined as *the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population* (NHMRC and MoH 2006), and harvested cells are not expected to be a major component of the diet and will be infrequently consumed. Therefore in the case of cultured quail cells in the present application, the total folate content does not raise concerns across any population groups.

Other vitamins

Harvested cells provide more thiamin than either conventional quail or chicken breast (at least 135% EAR, 18% of EAR and 17% of EAR respectively per serving, for men aged 19-50). A single serving provides a similar amount of riboflavin as conventional quail and more than a serving of chicken breast (harvested cells: at least 76% of EAR; conventional quail: 84% of EAR, chicken breast: 5% of EAR, for men aged 19-50). It provides a similar or greater amount of pyridoxine per serving compared to conventional quail or chicken breast (101% or 183% of EAR for harvested cells, depending on the culture media used, 111% of EAR in quail and 66% of EAR in chicken breast). A serving of harvested cells provides at least 98% of the EAR for niacin, while a serving of conventional quail and chicken breast provide 99% and 130% of the EAR for niacin respectively.

On a per 100 g basis, harvested cells contain more thiamin than either conventional quail or chicken breast, 0.5 mg/100 g compared to 0.1 mg/100 g in comparator meats. It has a similar niacin content to conventional quail, 6.8 mg/100 g and 6.6 mg/100 g respectively; with chicken breast containing 11 mg/100 g. It contains a similar amount of pyridoxine to conventional quail and chicken breast, 0.4 or 0.7 mg/100 g depending on the culture media used, 0.7 mg/100 g in quail and 0.51 mg/100 g in chicken breast. The riboflavin content of harvested cells is between that found in conventional quail and chicken breast, at least 0.28 mg/100 g, 0.51 mg/100 g and 0.04 mg/100 g respectively (Appendix-I A1.4).

Harvested cells contain small amounts of retinol and vitamin C, similar to conventional quail and chicken (Appendix-I A1.4).

No nutritional concerns are raised from the thiamin, riboflavin, pyridoxine, niacin, retinol or vitamin C content of harvested cells.

Minerals

Vow provided data on the mineral content of both harvested cells and conventional quail including calcium, chromium, copper, iodine, iron, magnesium, manganese, phosphorous, potassium, selenium, sodium, sulphur and zinc. The percentage of each mineral's EAR provided in a single serving of harvested cells was compared to that provided by a serving of conventional quail and chicken breast (Appendix-I A1.5).

Sodium

The sodium content of harvested cells is higher than conventional quail, 79 mg/100 g or 119 mg/100 g depending on the culture media used, compared to 45 mg/100 g in quail and 48 mg/100 g in chicken breast. The suggested dietary target (SDT[14]) for sodium is no more than 2000 mg/day for adults however the usual intake of a high proportion of the Australian

[14] The suggested dietary target (SDT) is a daily average intake from food and beverages for certain nutrients that may help in the prevention of chronic disease. (NHMRC and MoH 2006).

population (17-99%) exceeds the previous UL[15] for sodium of 2.3 g/day (Appendix-III A3.5). A single serving of harvested cells provides 12% or 18% of the sodium SDT depending on the culture media used; conventional quail provides 4% and a serving of chicken breast provides 3% of SDT, however as noted previously the suggested serving size for harvested cells is likely to be an overestimation. The sodium intake from consumption of harvested cells is further discussed in the Dietary Intake/Exposure Assessment section 4.3.4 and 4.3.5 below.

Iron

Harvested cells contain 0.6 mg/100 g iron using either culture media, compared to 1.3 mg/100 g in conventional quail and 0.3 mg/100 g in chicken breast. A serving of harvested cells provides slightly less iron than conventional quail (29% vs 39% EAR), but more than a serving of chicken (6% of EAR). The iron intake from consumption of harvested cells is further discussed in the Dietary Intake/Exposure Assessment Section 4.3.4 and 4.3.5 below.

Other minerals

Harvested cells provide more zinc and selenium, and a similar amount of phosphorous per 100 g compared to conventional quail or chicken breast. Harvested cells contain a similar or lesser amount of potassium to conventional quail and chicken breast depending on the culture media used. They provide a similar proportion of the daily requirements of potassium to conventional quail meat or chicken breast (Appendix-I A1.5). The copper content of harvested cells per 100 g is similar to quail. Chicken breast contains trace amounts of copper. No nutritional concerns were raised due to the levels of zinc, phosphorous, selenium or potassium in harvested cells.

Harvested cells, conventional quail and chicken breast contain trace amounts of calcium, chromium, iodine, manganese and magnesium and were not considered further (Appendix-I A1.5). Vow provided data on the sulphur content of harvested cells however no comparison data were available for conventional quail or chicken.

4.2.4 Effect of the harvested cells on absorption of nutrients

FSANZ considered whether harvested cells, at the expected consumption level, could interfere with the absorption of other nutrients. Vow undertook a literature search in five databases (Cumulated Index to Nursing and Allied Health Literature, Medline, Food Science and Technology Abstracts, Toxline, and Proquest Environmental Science Index). Vow did not identify any relevant publications.

FSANZ also undertook a literature search in Pubmed on 2 March 2023 to identify any relevant literature[16]. No studies were identified. In general anti-nutritional factors are associated with foods from plant material (Gemede and Ratta 2014) and therefore studies on anti-nutritional factors in cultured quail cells would not be expected.

[15] The sodium UL for adults of 2300 mg/day was replaced in 2017 with an SDT of 2000 mg/day as a review of data failed to identify a point at which the relationship between higher sodium and higher blood pressure did not occur (NHMRC and MoH 2006). A UL for children aged 1-18 remains between 1000-2300 mg/day.

16 Search terms: ("cultured quail" or "in vitro quail" or "in-vitro quail" or "lab-grown quail " or "cell-based quail" or "cultivated quail" or "cultured chicken" or "in vitro chicken" or "in-vitro chicken" or "lab-grown chicken" or "artificial chicken" or "cell-based chicken" or "cultivated chicken" or "cultured meat" or "in vitro meat" or "in-vitro meat" or "lab-grown meat" or "artificial meat" or "cell-based meat" or "cultivated meat") AND ("antinutrient" or "antinutritional" or "anti-nutrient" or "anti-nutritional" or "bioactive" or "biologically active" or "absorbed" or "absorption").

4.3 Dietary intake/exposure assessment

Dietary intake/exposure assessment provides an estimate of the magnitude, frequency and duration (where appropriate) of intake/exposure to the risk factors identified in this assessment.

4.3.1 Objectives

- Estimate potential consumption of the harvested cells, with comparisons to the substitute food where appropriate.
- Estimate the dietary intake of certain nutrients the harvested cells contain, considering both adequacy and safety of intake.
- Estimate the dietary exposure to any production input of interest that will remain in the harvested cells, however the hazard assessment did not identify any substances that required a dietary exposure assessment to be undertaken.

4.3.2 Methodology

Dietary intake/ exposure assessments require data on the concentration of the chemical of interest in the food requested and consumption data for the foods, usually collected through a national nutrition survey.

The dietary intake assessment was conducted to estimate the levels of chronic dietary intake of certain nutrients in harvested cells that were considered to be relevant following the nutrition risk assessment. Chronic dietary intake estimates are used to represent the long term, usually life-long, dietary intake for the population from the range of foods containing the chemical of interest.

The dietary intake assessment for this application was undertaken using FSANZ's dietary modelling computer program Harvest[17], along with deterministic calculations outside of Harvest. This was done for estimating nutrient intakes. A summary of the general FSANZ approach to conducting dietary intake assessments is on the FSANZ website. A detailed discussion of the FSANZ methodology and approach to conducting dietary intake assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009b). Nutrient intakes were also determined from published information from national nutrition surveys.

The serve size of the harvested cells, as noted by Vow, was 300 g, without other ingredients added. So the dietary intake assessment included an evaluation based on the nominated serve size, however it also included an assessment based on how people could eat the product in reality (e.g. such as in amounts similar to conventional meats). An evaluation was also undertaken of the realistic nature of the 300 g serve size. This was to evaluate whether conventional meats are consumed at the amount of 300 g, and what sort of percentile this amount represents. An evaluation was also undertaken regarding the serve size of a conventional quail, such as a whole bird purchased at a retail outlet.

[17] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

4.3.3 Food consumption data used and population groups assessed

The food consumption data used for the dietary intake assessments were:

- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), one 24-hour food recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS 2015a).
- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS), one 24-hour food recall covering 3,275 New Zealand school children aged 5-14 years, with 25% of respondents also completing a second 24-hour recall (MoH 2005).
- 2008–09 New Zealand Adult Nutrition Survey (2008 NZ ANS): a 24-hour recall of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. (MoH 2011a; MoH 2011b).

The design of these nutrition surveys and the key attributes, including survey limitations, are set out on the FSANZ website.

In this assessment, dietary intakes were estimated for 'consumers only' (e.g. consumers of foods containing chicken meat/ conventional quail/ other meats) where food consumption amounts were used in the assessment. Nutrition survey respondents who had no consumption of these foods were not included in the results presented. All results were weighted to make them representative of the respective populations.

For the chronic dietary intake assessment, one day of food consumption data from both of the New Zealand surveys were used, whereas the average of two days of data from the 2011-12 NNPAS was used for Australia. Two day average intakes better reflect longer term estimates of dietary intake and therefore are a better estimate of the chronic dietary intake. Where usual intakes of nutrients are presented for Australia, these were derived using the National Cancer Institute Method (NCI Method) which uses the two days of consumption data from the survey. Where usual intakes are reported for New Zealand, these were estimated using the Iowa State University method C-SIDE. All usual intakes are derived from foods and beverages, but do not include intakes from dietary supplements.

For the nutrient intake assessments, population subgroups were used for Australia and New Zealand according to Nutrient Reference Value (NRV)[18] age/sex groups. Details of the population subgroups are provided in (Appendix-III A3.1). The results from the New Zealand nutrition surveys were not reported exactly according to the NRV age group cut offs. Hence, adjustments were made when presenting New Zealand results in comparison to the NRV.

Other information that was considered as part of the assessment were from the FSANZ Consumer Insights Tracker (see SD3 at the 1st CFS for details) and other published literature.

4.3.4 Iron and sodium intake assessments

Being a new food there is no consumption data available for cultured quail cells in national dietary surveys. Due to no (for New Zealand) or limited (for Australia) consumption data on conventional quail meat in national dietary surveys, chicken meat consumption data was alternatively used for this assessment as the best comparator and as a proxy for an amount of harvested cells that could be consumed. This is also aligned with the nutrition risk assessment.

[18] Nutrient reference values, Australia and New Zealand: https://www.nrv.gov.au/

The nutrition risk assessment found that harvested cells contain higher concentrations of iron and sodium than chicken breast (Appendix-I A1.5). Hence, intake assessments were conducted for sodium and iron to see whether their intakes are at a safe level (e.g. below the UL) considering the usual intake levels from food and beverages (*baseline scenario*) and for some harvested cells consumption scenarios.

4.3.4.1 Scenarios assessed

Scenario 1: Consumers choose to eat the harvested cells at amounts equivalent to total longer term consumption of chicken meat, in addition to conventional meat including chicken (a conservative assumption). Consumption amounts at the mean and 90th percentile (P90) were derived. These amounts differ for each of the specific population groups assessed. The chicken consumption values used to represent the harvested cell consumption amounts are representative of a longer term consumption pattern taking into account chicken meat that is eaten as a serve in its own right as well as other amounts from mixed dishes such as in main meals (e.g. stews or curries) or other uses such as in sandwiches.

Scenario 2: Consumers choose to consume the harvested cells at a proposed high serve size of 300 g/day (high consumption as suggested by Vow) or at a more average serve size of 142 g/day (mean consumption as derived from chicken meat consumption data for Australia when reported as eaten as a piece or serve of chicken as part of a main meal) in addition to conventional meat including chicken (the worst case scenario). This scenario simulates when a consumer eats a single portion of cultured quail cells as the key meat component of a main meal.

Both scenarios incorporate a very small degree of double counting as consumption of conventional chicken meat is not removed before adding in consumption of harvested cells. This is a conservative assumption made as part of FSANZ's standard tiered approach to undertaking dietary exposure assessments, where further, more refined assessments that may remove baseline chicken meat consumption, can be undertaken if the first tier risk assessment results indicate the need for a more refined assessment.

4.3.4.2 Concentration data used

Sodium and iron concentration data for harvested cells were taken from the nutrition risk assessment (Appendix-I A1.5). The highest sodium concentration (118.7 mg/100 g) detected in the harvested cells was used for the sodium intake assessment considering the worst case scenario. The iron concentration (0.6 mg/100 g) was similar in harvested cells grown using the two sets of culture media. Usual nutrient intake levels from food and beverages and intakes from dietary supplements were sourced from the Australian and New Zealand national nutrition surveys (ABS 2015b; ABS 2015e; MoH 2003; University of Otago and MoH 2011; MPI 2018). For the iron intake assessment usual intake levels from food and beverages and intake from supplements (e.g. vitamin and mineral tablets) were considered as the baseline intake. Usual intake levels only from food and beverages were considered as the baseline intake for sodium and other the nutrients assessed.

4.3.4.3 Calculating the nutrient intakes

The intake of iron and sodium from harvested cells was estimated by multiplying the estimated consumption amount of harvested cells by iron and sodium concentration in the harvested cells, respectively. For *scenario 1*, mean and high (P90) consumption amounts of harvested cells were estimated using chicken consumption data for Australia and New Zealand representing a longer term consumption amount as explained in Section 4.3.5.1. For *scenario 2*, the mean consumption amount of harvested cells was estimated using chicken consumption data for Australia representing a serve or portion of meat as explained

in Section 4.3.5.2 and high consumption was considered at 300 g/day as noted in the application.

To estimate the total iron and sodium intake for *scenario 1* and *scenario 2*, the intake of iron and sodium from harvested cells was added to baseline mean and high intakes of iron and sodium, respectively. For the high intakes of iron or sodium, the highest reported percentile from the published usual intakes of iron/ sodium were used for each country: 95th percentile intakes for the Australian population and 90th percentile intakes for the New Zealand population. The differences in these calculations for iron and sodium intakes between scenarios 1 and 2 were the assumed consumption amounts, the derivation of which was explained in Section 4.3.4.1.

4.3.4.4 Assumptions and limitations

The dietary intake assessment was designed to calculate the most realistic estimate of dietary intakes for iron and sodium as possible. However, where significant uncertainties in the data and information exist, conservative assumptions were generally used to ensure that the estimated dietary intake is not an underestimation.

Assumptions made in the dietary intake/exposure assessments included:

- One serving size of harvested cells is 300 g/day as suggested by Vow. This serving size seems to be reflective of consumption amounts for high consumers (P95 consumption of conventional meats as derived from national nutrition survey data). Hence, a mean consumption amount (142 g/day) was also derived from chicken meat consumption data as explained in Section 4.3.5.2 which was assumed to represent a more typical serve size.
- Mean nutrient intake data from dietary supplements for iron is available only for Australia, therefore it was also used as the level of intake for New Zealand.
- No high baseline usual intake from food and beverages for sodium is available for New Zealand, therefore it was considered to be equal to double the mean baseline usual intake from food as per a standard equation used by FSANZ (FSANZ 2009b).

No consumption data is available for the New Zealand population aged 2-4 years. No baseline intake data is available for sodium for some of the New Zealand age/sex groups (e.g. 7-10 years and 15-18 years). Therefore no results were presented for these age/sex groups.

4.3.5 Results

4.3.5.1 Poultry meat consumption

Consumption of chicken meat for Australia and New Zealand was initially estimated because it was assumed that ‘consumers choose to eat harvested cells at amounts equivalent to total longer term consumption of chicken meat’ for *scenario 1*. For this estimation, the Harvest ‘raw commodity model’ was used that considers consumption data of chicken eaten ‘as is’ (i.e. as a piece on a dinner plate) and from mixed dishes, such as chicken in a sandwich, on a pizza, in stir fries etc. Consumption amounts for the other poultry meat types including conventional quail were also estimated for comparative purposes. The mean and P90 consumption, along with proportion of consumers, for a variety of poultry meats that were included in the nutrition surveys for Australia and New Zealand are shown in Table 3. The Australia and New Zealand populations had the highest mean and P90 consumption for chicken meat with the highest proportion of consumers compared to other poultry meats. The estimated mean and P90 consumption of goose meat and mutton-bird meat for the New

Zealand adults was deemed not reliable due to very low proportion of consumers. As well, it was noted that the mean and P90 consumption for the conventional quail meat for the Australian population is not reliable given the low proportion of consumers. These results also justify the conservative approach of considering chicken meat as the most appropriate comparator for harvested cells for this intake assessment.

The mean and P90 chicken meat consumption for several Australian and New Zealand population subgroups (according to the NRV age group) were also estimated (Appendix-III A3.2). These results were used when estimating iron or sodium intake from harvested cells for the respective population subgroups for *scenario 1*.

Table 3 *Poultry meat consumption for Australia and New Zealand**

Meat type	Australia**			New Zealand**					
	2 years and above			5-14 years			15 years and above		
	Consumption (g/day)		% cons to resp.#	Consumption (g/day)		% cons to resp.#	Consumption (g/day)		% cons to resp.#
	Mean	P90		Mean	P90		Mean	P90	
Chicken	94	190	61.8	90	192	41.1	127	262	38.7
Duck	53	101	<1	18	24	<1	91	253	<1
Goose	—	—	—	—	—	—	197	197	<1
Mutton-bird	—	—	—	—	—	—	714	714	<1
Ostrich	—	—	—	3	3	<1	—	—	—
Quail	38	52	<1	—	—	—	—	—	—
Turkey	49	120	1.7	23	50	1.5	37	71	2.7
Emu	—	—	—	—	—	—	—	—	—
Pigeon	—	—	—	—	—	—	—	—	—
Poultry, unspecified type	1	1	47.0	18	67	26.7	32	97	17.8
Grand Total	74	175	80.1	75	182	56.2	115	255	49.6

* Harvest raw commodity model was used for this estimation.
** Average of two days of consumption data for Australia and one day of data for New Zealand were used.
#Consumers as a % of total respondents.
— no data available in the nutrition surveys, i.e. not reported as consumed.

4.3.5.2 Assessment of the proposed serving size for the harvested cells and consumers' insights/perceptions on cell-cultured meat in Australia and New Zealand

A separate assessment was conducted to understand the serving size for harvested cells (300 g/day) proposed by Vow in comparison to the serving sizes of chicken and other meats (e.g. beef, lamb, pork, etc.) for Australia and New Zealand extracted from nutrition survey data. For this assessment, Day one consumption data for the three surveys were estimated using Harvest 'nutrient intake model' that considers consumption data where respondents reported meat eaten 'as is' (i.e. as a piece on a dinner plate). It does not include consumption amounts from mixed dishes such as meat on pizzas, on sandwiches or in casseroles. Therefore the consumption amounts derived using this method represent a portion size of meat, and the consumption amounts, which are derived from a distribution of consumption amounts from individuals, are not skewed down by the consumption of smaller amounts of meat from mixed dishes.

The results are shown in Table 4. The results revealed that the 300 g serving size for harvested cells mostly lies around the P95 consumption amount of chicken and other meat types for the Australian population (300-330 g) as well as the P95 chicken consumption for the New Zealand adult population (282 g). This indicates that the 300 g serving size would be a realistic consumption amount, it is within the normal consumption distribution for conventional meat, and it would be representative of a high consumer (P95). Accordingly, mean chicken meat consumption for the Australian population (142 g/day) was considered as the mean consumption for harvested cells for the nutrient intake assessment for *scenario 2*.

The consumption of conventional quail has been provided only for reference as the limited data from the nutrition survey does not allow the derivation of a reliable serving size. However, a literature search showed that the usual serving size of conventional quail (deboned) ranges from 85 g to 180 g (2 quail per serve)[19] and a dressed quail weighs from 80 g to 200 g[20] (Nasr et al. 2017). It was also noted that one quail has been estimated at 76 g in the AUSNUT 2011-13 nutrient composition database used for the Australian 2011-12 nutrition survey[21]. As noted in the nutrition risk assessment, Vow indicated that the 5th percentile weight of meat per quail is 160 g and the 95th percentile is 200 g.

Table 4 *Estimated meat consumption for Australia and New Zealand**

Meat type	Australia				New Zealand							
	2 years and above				5-14 years				15 years and above			
	Consumption (g/day)			% cons to resp.#	Consumption (g/day)			% cons to resp.#	Consumption (g/day)			% cons to resp.#
	Mean	P90	P95		Mean	P90	P95		Mean	P90	P95	
Chicken	142	268	330	17.1	110	195	239	21.4	132	232	282	21.2
Quail	68	76	76	<1	—	—	—	—	—	—	—	—
Beef	160	246	300	11.7	140	284	390	19.5	181	405	541	22.5
Lamb and mutton	133	210	312	5.4	101	179	252	4.5	137	282	350	6.8
Pork	123	232	300	4.9	68	145	189	18.8	104	232	331	23.2

* Harvest nutrient intake model was used for this estimation. One day data from the three surveys was used.
#Consumers as a % of total respondents.
— no data available in the nutrition surveys.

The preliminary findings of the FSANZ Consumer Insights Tracker (CIT), an online survey of 1237 Australian and 810 New Zealand consumers aged 18+ years (see SD3 at the 1st CFS) for an overview of the methods, results and limitations of the CIT), reported that only 23.6% of consumers said they would include cell-cultured meat in their diet. Most of them (50.5%) said cell-cultured meat would partially replace traditional meat (e.g. farm-raised beef, chicken or pork), 14.3% of them said cell-cultured meat would completely replace the traditional meat and 37.5% of them said cell-cultured meat would be consumed in addition to the traditional meat. A consumers' perception survey conducted in New Zealand reported that out of 572 respondents aged 25–55 years who were meat consumers, 30% were willing to purchase (regularly, often, or always) cell-cultured meat instead of conventional meat (Giezenaar et al. 2023).

[19] Quail - boneless Nutrition Facts | Calories in Quail - boneless (checkyourfood.com); Quail, raw, meat only, breast nutrition facts and analysis. (nutritionvalue.org)
[20] Game Birds - Poultry Hub Australia
[21] AUSNUT 2011–13 food measures database file (foodstandards.gov.au)

4.3.5.3 Iron intake

Estimated dietary intakes of iron for the Australian population are presented in Table A3.3 Appendix-III. The mean and high (P95) usual intakes of iron at baseline did not exceed the UL for all the Australian population subgroups assessed. However, a high proportion (about 38-40%) of females aged from 14-50 years had inadequate iron intake (i.e. <EAR) at baseline. The results showed that the total high intake of iron did not exceed the UL for all the Australian population subgroups even for the worst case scenario assessed (*scenario 2*).

Estimated usual dietary intakes of iron for the New Zealand population are shown in Table A3.4 Appendix-III. The mean and high (P90) usual intakes of iron did not exceed the UL for all the New Zealand population subgroups assessed. The proportion of New Zealand children with inadequate iron intake was 6.6% at baseline (based on UK Dietary Reference Values) (MoH 2005). The prevalence of inadequate iron intake was high among New Zealand females aged 15-18 years at baseline (34.2%) (MoH 2011a; MoH 2011b). The total high intake of iron did not exceed the UL for all the New Zealand population subgroups even for the worst case scenario assessed (*scenario 2*).

4.3.5.4 Sodium intake

Estimated mean and high dietary intakes of sodium for the Australian population are presented in Table A3.5 Appendix-III. The mean dietary intake of sodium at baseline exceeded the UL or current SDT (Suggested Dietary Target) for most of the Australian population subgroups except females aged 51 years and older. The high dietary intake of sodium at baseline exceeded the UL or current SDT for all the population subgroups assessed. It was found that a large proportion of the Australian population (24-99%) aged 70 years or below exceeded the UL for sodium and the proportion was highest for the young population aged 18 years and below (51% or higher), noting that the NRVs have since been revised and a UL is no longer established for adults. The previous ULs for adults were 2300 mg/day, whereas the new SDT is 2000 mg/day, therefore there would be a greater proportion exceeding the current SDT. ULs are still in place for children up to 18 years and range between 1000-2300 mg/day depending on the age group.

For *scenario 1*, total mean dietary intake of sodium exceeded the UL or current SDT for adults for most of the Australian population subgroups except females aged 71 years and older whereas total high dietary intake of sodium exceeded the UL or current SDT for adults for all of the Australian population subgroups assessed. However, increase in total mean and high dietary intake of sodium in comparison to the mean and high baseline intake, respectively, was at or below 7%.

For *scenario 2*, total mean and high dietary intake of sodium exceeded the UL or current SDT for adults for all of the Australian population subgroups assessed. For this scenario, increase in total mean and total high dietary intake of sodium (in comparison to the mean and high baseline usual intakes, respectively) was at or below 12% and 19% respectively. The increase in high intake was highest for the 2-3 year age group. However, it is highly unlikely that this population subgroup would eat the estimated amount of harvested cells per day as assumed for the *scenario 2*, the worst case scenario (high of 300 g/day and mean 142 g/day).

Estimated mean and high dietary intakes of sodium for the New Zealand population are shown in Table A3.6 Appendix-III. The mean and high usual intake of sodium at baseline exceeded the UL or current SDT for adults for all of the New Zealand population subgroups assessed.

For *scenario 1*, total mean and high dietary intake of sodium exceeded the UL or SDT for all of the New Zealand population subgroups assessed. However, increase in total mean and high dietary intakes of sodium in comparison to the mean and high baseline usual intake, respectively, was at or below 7%.

For *scenario 2*, total mean and high dietary intake of sodium exceeded the UL or SDT for all of the New Zealand population subgroups assessed. Increase in total mean and high dietary intakes of sodium in comparison to the mean and high baseline usual intakes, respectively, was at or below 9% for the New Zealand population.

4.3.5.5 Assessment of estimated usual dietary intakes of other nutrients

According to the nutrient compositional analysis conducted comparing harvested cells, conventional quail and chicken meat, assessment of estimated usual intake from food and beverages (intakes at baseline) of some other nutrients such as proteins, dietary fats and selected vitamins was of interest. For this assessment, published usual intake data for Australia and New Zealand were analysed. Data from other countries were considered when there were no or limited data available for Australia and New Zealand.

Protein

Estimated usual dietary intakes of protein for Australia and New Zealand are provided in Table A2.1 and A2.2 Appendix-II, respectively. The results showed that less than 1% of each of the Australian population subgroups assessed aged 70 years or below had usual protein intakes less than the EAR at baseline. This proportion was higher for the 71 years and older age group (male 13.7% and female 3.8%) as a result of their EAR being higher than other adults. The proportion of inadequate intake of protein was also higher for the 71 years and older population subgroup in New Zealand (male 13.4% and female 15.5%).

Dietary fat

Estimated usual dietary intakes of selected dietary fats and fatty acids for the Australian population are given in Table A2.3 Appendix-II. The results showed that mean or high (P95) intake of long-chain omega 3 fatty acids (DHA, EPA, DPA) did not exceed the ULs for all of the Australian population subgroups assessed. Estimated usual dietary intake of polyunsaturated fat and saturated fat for the New Zealand population is given in Table A2.4 Appendix-II.

Vitamins

Estimated usual dietary intakes of cobalamin (vitamin B_{12}) and dietary folate equivalents (DFEs) for Australia and New Zealand are provided in Table A2.5 and A2.6 Appendix-II, respectively.

The proportion with usual intake of cobalamin less than the EAR was below 1% for Australian males. However, this was higher (above 5%, up to 8.3%) for the Australian females aged 14 years and older. Most of the New Zealand females aged 19 years and older had higher inadequate intake of cobalamin and this was highest among the 71+ females age group (27%). There is insufficient data to establish ULs for cobalamin, and there is no evidence to suggest that current intakes from foods and dietary supplements represent a health risk (NHMRC and MoH 2006). High (P95) usual intakes of cobalamin ranged between 4.8 and 8.4 µg/day across all of the Australian population groups assessed.

In terms of usual intake of dietary folate equivalents, a higher proportion of Australian females aged 14 years and older had usual intake less than the EAR (above 6%, up to 11%) whereas it was below 3% for Australian males aged 9 years and older.

Usual dietary intake of biotin (vitamin B_7) has been estimated for the New Zealand population using a food data base developed by DSIR (Department of Scientific and Industrial Research), in New Zealand (LINZ 1992). The estimated median intakes were 37.9 µg/day for males aged 15–18 years, 26.7 µg/day for females aged 15–18 years, 33 µg/day for males 19 years and over and 27 µg/day for females 19 years and older. There are no population intake data for Australia (NHMRC and MOH 2017). There are no EARs or ULs established for biotin. It has been reported that mean biotin intake from food and beverages in some western populations is about 35–70 µg/day[22]. In Japan, mean usual intakes for males and females were 52.1 µg/day and 47.5 µg/day, respectively (Imaeda et al. 2013). Mean and P95 usual intakes of biotin were reported at 44.656 µg/day and 169.416 µg/day for Korea (Kim et al. 2011).

[22] Biotin - Health Professional Fact Sheet (nih.gov)

4.4 Harvested cultured quail cell specifications

4.4.1 Microbiological specifications

Vow developed microbiological specifications (Table 5) based on microbiological standards for RTE foods which meet or exceed those in the Code (Standard 1.6.1 and S27—4; FSANZ 2022), and included coliforms as an indicator organism. They propose to apply the specifications to the harvested cell biomass, with any results exceeding the limits resulting in batch failure.

Vow included *Salmonella* as it is associated with animals including poultry (though typically associated with the gastro-intestinal tract) and their environment. Key risk factors of *Salmonella* contamination in the harvested cells includes a high level of *Salmonella* spp. contamination in the raw ingredients, incorrect time and temperature combinations applied during handling, particularly post-harvest and poor equipment or personnel hygiene.

Vow included *Staphylococcus* (<10 cfu/g) and *L. monocytogenes* (not detected in 25 g) in their testing of harvested cells due to the potential for their presence. Vow did not include them in their proposed specifications following nil detection for all tests on several batches of harvested cells. Based on their results and use of good practices, Vow considers these microbiological contaminants are not likely to be present in the harvested cells. They will instead monitor them via their environmental monitoring given coagulase positive staphylococci and *L. monocytogenes* can potentially be introduced through the manufacturing process.

Vow demonstrated the microbiological specifications were met on product sampled from consecutive batches (n=5). Noting these batches are at volumes less than expected future production levels, FSANZ expects ongoing microbiological testing at commercial production will demonstrate the specifications are met. Vow will microbiologically test each batch in-house, with independent NATA-accredited laboratory testing on every 20th batch to determine these parameters are met. Vow advised any batches that do not meet the required microbiological parameters will be discarded.

Table 5 *Proposed microbiological specification by Vow*

Parameter tested	Proposed specification
Standard plate count (CFU/g)	<10^4
Coliforms cfu/g	<100
E. coli (MPN/g)	<3
Enterobacteriaceae (cfu/g)	<100
Salmonella	Not detected in 25 g

FSANZ considered Vow's proposed specifications and specifications for other potentially hazardous foods, the product's potential to support microbial growth, that this is a new food type and production system, and that further processing steps were not assessed in this application.

FSANZ's microbiological hazard assessment of the production and harvesting processes and of the harvested cells identified the potential for microbial contamination was greatest during harvest and post-harvest handling. Microbial contamination can enter food from food contact surfaces, equipment and personnel; however, other potential sources of microbial contamination should not be excluded. Microbiological specifications should be used to monitor the performance of a process or a food safety control system at a specified point in the food chain, using a robust sampling and testing program (Codex 1997).

Microbiological specifications should include both microbial indicator organisms (process control) and food-associated pathogens (safety) for the harvested cell biomass. Other measures including additional microbiological testing are included under Vow's HACCP plan. Given the lack of data for this food type and production system, applying specifications to the harvested cells is supported at this time.

Summary of potential microbiological hazards

As noted in Section 2.2.1, the risk of foodborne illness arising from the original cell line is low, and ingress of pathogens is also unlikely to occur during the expansion phase when good practices are effectively implemented. These hazards can be managed through application of an appropriate food safety program which includes a HACCP-based food safety plan supported by GHP, GMP and GCCP.

Indicator organisms

In-process monitoring should be used to confirm microbiological contamination as recommended by FAO/WHO (2023). In Vow's proposed microbiological specifications listed in Table 5, standard plate count (SPC) is used as an indicator of overall hygienic processing while coliforms, *E. coli* and Enterobacteriaceae are indicators of potential faecal contamination (NRC 1985; FSANZ 2022). FSANZ notes cell expansion is occurring in a closed aseptic system that should prevent the ingress of any microorganisms. As such, presence of microorganisms should be as close to nil detect as possible.

Comment on final product of this assessment

In this application FSANZ assessed to the point of freezing the harvested cell biomass (i.e. harvest, centrifugation and initial freezing). Microbiological safety of the harvested cells will be reliant on consistent implementation of through-chain control of microbiological hazards, storage conditions, and preparation of the harvested cells by food service/consumers; these factors all influence the microbiological safety outcome of the final product.

4.4.2 Compositional specifications

As the Vow cultured quail cells are a new food not granted permission under any jurisdiction, there is no specification or published primary or secondary source for a specification in section S3—3 of the Code, nor is there a Codex Standard for foods produced using cell culture technology. FSANZ is proposing to insert a new standard in the Code (Standard 1.5.4 – Cell-cultured foods), and products will be required to comply with Schedule 3 – Identity and purity.

The proposed specification will include quantitative parameters based on the information provided. Vow has proposed specifications along with evidence of adherence to these. The protein specification for harvested cells of >4 g/100 g reflects the alternative quantification method used by Vow, however the validated laboratory analysis indicates a protein content of 9 g/100 g. Table 6 shows the proposed compositional parameters based on updated data subsequent to that in the application, whilst allowing for production variation. Vow provided data from multiple production batches to demonstrate compliance to these parameters.

Table 6 *Proposed specification (compositional only)*

Parameter	Specification
Protein (%)	not less than 4
Moisture (%)	not less than 80
Ash (%)	not more than 1.5
Fat (%)	not less than 0.5 and not more than 3.0
Carbohydrates (%)	not more than 1

4.4.3 Heavy metal analysis

Vow provided test results using ICP-MS (Inductively coupled plasma mass spectrometry) for heavy metals for multiple batches of cultured quail cells; antimony (<0.01 mg/kg), arsenic (<0.05 mg/kg), cadmium (<0.01 mg/kg), lead (<0.01 mg/kg), mercury (<0.01 mg/kg), and tin (<0.02 mg/kg). These levels are below the additional supplementary requirements for metals in S3—4 of the Code: arsenic (1 mg/kg), cadmium (1 mg/kg), lead (2 mg/kg) and mercury (1 mg/kg).

4.5 Harvested cells conclusions

Microbiological hazard assessment

Hygienic practices are essential when handling harvested cells as they will be vulnerable to contamination and readily support microbial growth. The HACCP-based approach should include a robust monitoring regime. In addition to the specifications Vow proposed, environmental and product testing for *Listeria* should also be included in their monitoring. In addition to Vow's proposed *Salmonella* specification, a *L. monocytogenes* criteria may be required for this new food which has no previous history of use. *L. monocytogenes* has been identified as a medium to high risk foodborne pathogen that can enter the food during harvesting and final processing of the cell biomass. It can grow at refrigeration temperatures and cause severe illness. Testing for coagulase positive staphylococci, yeasts and mould are also recommended for indicators of process hygiene failure. As the bioreactor is a sterile production unit, presence of microorganisms should be as low as possible.

Nutrition and dietary intake assessment

The nutrition risk assessment and dietary intake assessment determined if the consumption of harvested cells would cause a nutritional imbalance in the diet, comparing nutrient content per serving of harvested cells (300 g) with a serving of chicken breast or conventional quail. No nutritional issues were identified for the majority of nutrients assessed. More detailed evaluations were undertaken for some specific nutrients found to be present at high levels.

The harvested cells contain less protein than either conventional quail or chicken breast. As most Australian and New Zealanders consume sufficient protein in their diet, with less than 1% of the Australian population aged up to 70 years having usual protein intake less than the EAR at baseline, and the harvested cells not expected to be a large contributor to the diet, the protein content does not raise a nutritional concern. The proportion of the population with inadequate intakes of protein at baseline was higher for the 71 years and older population subgroup in Australia and New Zealand as a result of their EAR being higher than other adults. However given the small contribution the harvested cells will make to the diet, this is not considered to be a concern for this population group either.

The total and polyunsaturated fat content of the harvested cells is similar to chicken breast and is not a nutritional concern. The mean or high (P95) usual intake of long-chain omega 3 fatty acids (DHA, EPA, DPA) did not exceed the ULs for all of the Australian population subgroups assessed at the baseline.

Overall the vitamin content per serving of harvested cells is similar to or higher than conventional quail. No nutritional concern exists for the thiamine, riboflavin, pyridoxine, niacin, retinol or vitamin C content of the harvested cells.

The harvested cells contain high levels of cobalamin and biotin, resulting in intakes up to 929 times the EAR and 9 times the AI respectively per serving. However, no ULs have been set and recent studies do not report adverse effects from intake at high levels. Therefore, combined with the expected infrequent consumption of the harvested cells, a nutritional risk is not expected due to the consumption of cobalamin or biotin present in the cells. At the baseline, the proportion with usual intake of cobalamin less than the EAR was high for Australian females aged 14 years and older (up to 8.3%) and most New Zealand females aged 19 years and older (up to 27%). The highest estimated mean usual intakes of biotin was 37.9 µg/day for males aged 15 years and older and 27 µg/day for females 15 years and older in New Zealand (LINZ 1992); no data were available for Australian populations.

Based on Vow's suggested serving size of 300 g of harvested cells, the total folate content per serving if present as folic acid would reach the UL for individuals aged 14-18 years, if cells were grown with culture media 1. Due to the expected infrequent consumption and likely overestimated serving size of harvested cells, no nutritional concern is expected from folate intake.

In most cases the mineral content of the harvested cells is similar to conventional quail and chicken breast. No nutritional concerns were raised for the zinc, phosphorous, selenium or potassium content of the harvested cells.

The content of some nutrients that were considered to be relatively high in harvested cells were considered in Section 5 Hazard/Risk Characterisation.

5 Hazard/Risk characterisation

The hazard and risk assessment addressed microbiology, biotechnology, toxicology, allergenicity, nutrition and dietary intake/exposure considerations. As documented in this report, no safety concerns were identified in the biotechnology, toxicology and allergenicity evaluations based on the information available as part of this application. Vow analysed for the presence of gluten in the harvested cells due to potential carry over of barley proteins from the cell culture medium. Levels were below the limit of detection. Potential hazards identified in the microbiological and nutrition assessments are discussed below.

5.1 Microbiological hazard characterisation

5.1.1 *Listeria monocytogenes*

L. monocytogenes is ubiquitous in the environment and has been isolated from domestic and wild animals, birds, soil, vegetation, fodder and water. It can become established in food processing environments, on both food and non-food contact surfaces including the floors, drains and wet areas of food processing factories (Dos Santos et al. 2021; Ferreira et al. 2014; Olsek et al. 2022; FSANZ 2013). Once in the processing environment, *L. monocytogenes* can persist and resist standard cleaning. Microbiological contamination of food products can occur through poor hygienic practices of food handlers; or by exposure of

food ingredients to contaminated air, water, raw materials or food-contact surfaces (Codex 2007).

While *L. monocytogenes* generally doesn't affect healthy people, for susceptible populations, *L. monocytogenes* can cause severe disease that is potentially life threatening, with a case fatality rate of 15-30%. Published data indicate that contaminated foods responsible for foodborne listeriosis usually contain levels of *L. monocytogenes* >100 cfu/g (Ryser and Buchanan 2013). In 2019, the European Commission applied a criteria of <100 cfu/g for RTE products during their shelf life, whether or not they support growth; and, for RTE foods that support growth, absence in 5 x 25g samples at the point they leave control of the food producer.

Listeria is identified as a potential hazard that could be introduced during the harvesting and post-harvest stages of production of cultured quail cells. *L. monocytogenes* is able to grow in food at refrigeration temperatures down to 5°C, albeit slowly. Due to a lack of natural flora present within the cell biomass there is the potential for pathogenic organisms such as *L. monocytogenes* to achieve a cell density higher than conventional meat during chilled storage (Jia et al. 2020).

Due to the severity of illness, the potential for growth and the lack of critical control points applied during production of the cell biomass, *L. monocytogenes* in cell-cultured quail is considered to pose a medium to high risk to public health and safety. *L. monocytogenes* is controlled through an effective heat treatment. Vow advises the final product will be 'cooked' however this stage was not assessed as part of this application.

5.1.2 *Salmonella*

At least 2500 serotypes of *Salmonella* spp. have been identified, which differ in their reservoir host, growth characteristics and severity of disease they cause. Some serotypes are host-specific, some are host-adapted, while others have a broad host range (Jay et al. 2003; Wallis 2006; FSANZ 2020). *Salmonella* spp. are transmitted by the faecal-oral route, through consumption of contaminated food and water or from direct contact with infected people and animals (Jay et al. 2003). Ruminants are a main reservoir of *Salmonella* spp. although some strains have been linked to chickens and eggs. As there is no evidence to indicate *Salmonella* spp. were a risk from the initial embryonic cells sourced from eggs to establish the cell line, it is unlikely *Salmonella* would be present in the harvested product. If contamination occurred, the source would most likely be from personnel infected with *Salmonella*, and (mis)handling the harvested cells. Growth of *Salmonella* spp. can occur at temperatures ranging between 5.2 – 46.2°C and where the pH is in the range of 3.8 – 9.5. *Salmonella* spp. can survive for months or even years in low moisture foods and are able to survive frozen storage at -20°C. Should *Salmonella* contamination occur at harvesting or post-harvesting, it will be able to survive and potentially grow in the biomass depending on its storage temperature. Good hygienic practices to prevent cross-contamination would prevent *Salmonella* being introduced during handling by personnel.

5.1.3 *Staphylococcus*

Humans are the main source of coagulase positive staphylococci (e.g. *St. aureus*) where they are normally present on skin and nasal passages. Infected food handlers are a significant source of food contamination which can occur either by direct contact or indirectly via skin fragments or respiratory droplets. Environmental sources tend to be located in hard to clean/sanitise areas that can include food-processing equipment or non-food contact surfaces such as ventilation systems. Illness occurs via ingestion of toxin(s), which reach intoxication levels when the concentration of *Staphylococcus* exceeds 10^5 per gram. Growth of *St. aureus* can occur at temperatures ranging between 10 – 45°C, with a pH in the range

of 4.8 – 9.0, and with a water activity of higher than 0.85. *St. aureus* does not compete well with other bacteria, but would be able to grow in the microbiologically naïve cell biomass depending on the storage temperature.

5.1.4 Scale of production

The microbiological hazard assessment was performed on harvested cells produced in amounts which are smaller than the expected final production size. The assessment also considered up to the frozen harvested cells stage before any further processing. Vow's microbiological specifications and those identified by FSANZ are based on data at this scale of production. As the size of production and the number of times draw down and media replenishment increases, opportunity for microbial hazards entering the process and the product also increases. Microbiological outcomes are not constant and will fluctuate depending on a wide range of production factors, and will rely on appropriate hazard management being implemented. Ongoing monitoring against and adherence to microbiological criteria identified in Vow's specifications and/or HACCP plan should be demonstrated when larger production scales are used, to ensure the harvested cells continue to meet microbiological specifications.

It is not possible to fully characterise the risk at commercial production scale due to the uncertainty associated with elements of production process that would influence the microbiological outcomes and therefore any associated risk to public health. Adherence to a HACCP-based food safety system with supporting good practices should continue to mitigate identified hazards.

Vow advises the frozen harvested cell biomass will be thawed and further processed into the final food product which is subject to a 'cook' step. The harvested cells and likely the final food both have the potential to support microbial growth with *L. monocytogenes* the most likely pathogen. As the microbiological status or processing of the harvested cells into the final food is unknown and has not been assessed, *L. monocytogenes* should be managed during the production, harvest, packaging and storage stages. Given the potential severity of illness there should be ongoing monitoring of the production environment and the final food product.

5.1.5 Other hazards

Foodborne pathogens, including faecal-associated pathogens such as *Salmonella, E. coli,* and skin-associated coagulase positive staphylococci are potential hazards that could contaminate the cell biomass during further processing either from personnel or other ingredients. Up to this point, the cells have not undergone any microbiological control step other than the use of good hygienic practices to manage these hazards up to harvesting cells, so a recognised microbiological control step (e.g. cooking) should be applied before consumption to ensure the safety of the final food. Vow has indicated this will occur.

It is unlikely the identified microbial hazards will change with increased production scale but their management may need to be reassessed, using a HACCP/food safety plan, to ensure the product meets Vow's identified microbial specifications as well as those identified by FSANZ. Data generated from ongoing monitoring against microbial specifications and process monitoring will enable full risk characterisation.

5.2 Nutrition risk characterisation – iron and sodium

Harvested cells contain higher concentrations of iron and sodium than chicken breast. The risk characterisation for iron and sodium involved comparing their dietary intake estimates for different population groups in Australia and New Zealand with NRVs.

At the highest reported baseline levels of iron intake, no age/sex groups assessed in Australia and New Zealand exceed their respective ULs. The total high intake of iron did not exceed the ULs for the Australian and New Zealand populations even if consumers eat a 300 g of the harvested cells daily in addition to conventional meat.

The mean and high usual intake of sodium at baseline exceeded the ULs or SDTs for all of the population subgroups assessed for Australia and New Zealand, except the mean usual intake for females aged 51 years and older. If consumers eat harvested cells at a 300 g/day (high) or at a 142 g/day (mean consumption) in addition to conventional meat including chicken (*scenario 2*), the increase in total mean and high dietary intake of sodium (in comparison to the mean and high baseline usual intake, respectively) was at or below 9% for the New Zealand population groups assessed. Increase in total mean dietary intake of sodium was at or below 12% for the Australian population groups assessed and this was highest for the 2-3 years age group. Increase in total high dietary intake of sodium (in comparison to the high baseline usual intake) ranged from 8% to 19% for the Australian population and this was highest for the 2-3 years age group. The highest increase (19%) would be reduced to 12% if the lower sodium concentration detected in the harvested cells was used for the intake assessment. However, it is unlikely that 2-3 year olds would eat 300 g (high) or 142 g (mean) of the harvested cells every day as assumed for *scenario 2*. Hence, it is unlikely that consumption of the harvested cells would pose an additional risk in addition to that from current sodium intakes for the Australian and New Zealand populations.

Additionally, the studies conducted on consumers' insights/perceptions about cell-cultured meat (e.g. the harvested cells) indicated it is unlikely that consumers would eat cell-cultured meat in the same way they currently eat conventional meat, or the majority of people would eat cell-cultured meat in addition to conventional meat (see Section 4.3.5.2). Therefore, the assumptions used in the intake assessments for this application (e.g. consumption of harvested cells in the same way they eat chicken in addition to conventional meat including chicken) would result in overestimation of dietary intakes (e.g. sodium intake).

Overall, considering all of the evidence there were no nutritional risks identified from the consumption of the harvested cells containing the levels of nutrients provided in the application, particularly given the likely infrequent consumption of the harvested cells.

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Appendix-I Nutrient content comparisons

Table A1.1. Essential amino acid content of the harvested cells compared to quail meat sampled in Australia and Singapore provided by Vow

Amino acid	Harvested cells, media 1	Harvested cells, media 2	Quail Australia	Quail Singapore	Chicken, light and dark meat[+]
	g/100 g protein				
Histidine	4.2 ± 0.12	1.7 ± 0.02	3.2 ± 0.01	2.64 ± 0.03	4.13
Isoleucine	4.9 ± 0.04	3.7 ± 0.16	5.3 ± 0.43	4.51 ± 0.07	6.44
Leucine	9 ± 0.07	7 ± 0.23	8.8 ± 1.7	7.55 ± 0.14	10.15
Lysine	8.5 ± 0.45	8.3 ± 0.16	12 ± 1.1	8.25 ± 0.37	11.67
Methionine	2.3 ± 0.31	2 ± 0.04	3.2 ± 0.22	2.67 ± 0.03	2.97
Phenylalanine	4.8 ± 0.05	3.4 ± 0.1	4.5 ± 0.41	3.75 ± 0.08	5.03
Threonine	4.8 ± 0.02	3.9 ± 0.1	5.2 ± 0.43	4.19 ± 0.07	5.90
Tryptophan	1.6 ± 0.04	1.4 ± 0.07	0.93 ± 0.11	Not measured	1.64
Valine	5.7 ± 0.05	4.3 ± 0.17	5.6 ± 0.66	4.59 ± 0.08	6.77

[+] Data from Fox et al. (1988)

Table A1.2. Fat content of the harvested cells, conventional quail meat and chicken breast

Fat type	Harvested cells, media 1	Harvested cells, media 2	Quail, lean flesh and skin, raw*	Chicken, breast, lean flesh, raw*	Harvested cells, media 1	Harvested cells, media 2	Quail	Chicken breast, lean flesh, raw
	per 100 g				per 300 g serving		per 180g serving	Per 142 g serving
Total fat (g)	1.5	1.63	11	0.8	4.5	4.9	19.8	1.14
Total saturated (g)	0.63	0.67	3.3	0.25	1.89	2	5.94	0.36
Total monounsaturated (g)	0.81	0.92	4.52	0.31	2.44	2.76	8.14	0.44
Total polyunsaturated (g)	0.01	0.01	2.57	0.16	0.04	0.04	4.63	0.23
Total trans fatty acids (mg)	40.5	31	62.4	3.78	121.5	93	112.3	5.37

* Data from Australian Food Composition Database (FSANZ 2023)

Table A1.3. Essential fatty acid content of quail and chicken breast

Fatty acid	Chicken breast, raw per 100g	Quail per 100g	AI~ men 19+/day	% AI Quail per 180g serving	% AI Chicken breast per 142 g serving
Linoleic acid (g)	0.11	2.23	13 g	30.88	1.20
Linolenic acid (g)	0.01	0.17	1.3g	23.54	1.09
EPA (mg)	3.78	0	-	-	-
DPA (mg)	6.05	0	-	-	-
DHA (mg)	3.78	31.18	-	-	-
Total EPA, DPA and DHA (mg)	13.61	31.18	160 mg all 3.	35.08	12.08

~ Adequate intakes (AI) for men aged 19+ from National Health and Medical Research Council Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes in New Zealand Ministry of Health editor. Australian Government, Department of Health and Ageing.

Table A1.4. Vitamin content of the harvested cells compared to quail and chicken breast

Vitamin	Harvested cells, media 1 per 100 g	Harvested cells, media 2 per 100 g	Quail, lean flesh and skin, raw* per 100 g	Chicken, breast, lean flesh, raw * per 100 g	EAR~ men 19-50 per day	% EAR harvested cells, media 1 per 300 g serving	% EAR harvested cells, media 2 per 300 g serving	% EAR quail per 180 g serving	% EAR chicken breast per 142 g[$] serving
Retinol[#] (µg)	< 5	< 5	20	4	625	0	0	5.8	0.9
Thiamin (mg)	0.5	0.45	0.1	0.12	1.0	150	135	18.0	17.0
Riboflavin (mg)	0.28	0.37	0.51	0.04	1.1	76	101	83.5	5.2
Niacin (mg)	6.8	3.9	6.6	11	12.0	163	98	99.0	130.2
Pyridoxine (mg)	0.67	0.39	0.68	0.51	1.1	183	106	111.3	65.8
Cobalamin (µg)	619	457	1.2	0.1	2.0	92,850	68,550	108.0	7.1
Vitamin C (mg)	<1	<1	0	0	30.0	0	0	0.0	0.0
Biotin (µg)	87.3	86	5.6^	2.1	%AI30.0	%AI 873	%AI 860	%AI 34.0	%AI 9.9
Total folate (µg)	268	217	0	0	320[@]	251-502[+]	200-400[+]	0	0

* Data from Australian Food Composition Database (FSANZ 2023)

[#] Retinol equivalents

~ Estimated Adequate intake (EAR) from National Health and Medical Research Council Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes in New Zealand Ministry of Health editor. Australian Government, Department of Health and Ageing. Adequate intakes (AI) provided where an EAR could not be provided

[$] based on mean chicken meat Australian consumption data (g/day) for individuals age 2 years and above

^Mean data from Vow for conventional quail. No data available from Australian Food Composition Database

[@]EAR folate as dietary folate equivalents

[+]Percentage EAR calculated from total folate, provided as a range to account for either folic acid or natural folate

Table A1.5. Mineral content of the harvested cells compared to quail and chicken breast

Mineral	Harvested cells, media 1	Harvested cells, media 2	Quail, lean flesh and skin, raw*	Chicken, breast, lean flesh, raw*	EAR~ men aged 19-50 per day	% EAR harvested cells, media 2	% EAR harvested cells, media 1	% EAR quail lean flesh and skin, raw	% EAR chicken, breast, lean flesh, raw
	per 100 g					per 300 g serving		per 180 g serving	per 142 g[$] serving
Calcium (mg)	1.1	0.8	6	4	840	0.3	0.4	1.3	0.7
Chromium (µg)	<0.05	<0.05	0.1[#]	1.5[@]	AI 35	%AI 0	%AI 0	%AI 0.5	%AI 6.0
Copper (mg)	0.1	0.1	0.1	0.02	AI 1.7	%AI 17.6	%AI 22.9	%AI 10.6	%AI 1.7
Iodine (µg)	1.5	1.4	0.3	0	100	4.2	4.5	0.5	0
Iron (mg)	0.6	0.6	1.3	0.3	6	30	28.5	39	6.2
Magnesium (mg)	14	12	24	31	330-350[&]	10.3	12	12.3	12.6
Manganese (µg)	21	13	0	0	AI 5500	%AI 0.7	% AI 1.1	% AI 0	% AI 0.2
Phosphorus (mg)	232	173	210	240	580	89.5	120	65.2	58.8
Potassium (mg)	408	186	500	390	AI 3800	%AI 14.7	% AI 32.2	% AI 23.7	% AI 14.6
Selenium (µg)	23	22	15	17	60	110	115	45	40.2
Sodium (mg)	118.7	79	45	48	SDT[+] 2000	% SDT 11.9	% SDT 17.8	% SDT 4.1	% SDT 3.4
Zinc (mg)	1.3	1.5	0.7	0.5	12	37.5	32.5	11.1	6.2

* Data from Australian Food Composition Database, unless otherwise stated.
~ Estimated Adequate intake (EAR) from National Health and Medical Research Council Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes in New Zealand Ministry of Health editor. Australian Government, Department of Health and Ageing. Adequate intakes (AI) provided where an EAR could not be provided
[$] based on mean chicken meat Australian consumption data (g/day) for individuals age 2 years and above
[#] data provided by Vow for Australian Coturnix
[@] data from 22nd Australian total diet study (FSANZ 2008) or the "middle bound" mean of samples, derived by assigned a value of half the level of reporting to results that are less than the level of reporting
[+]SDT Suggested dietary target for sodium of 2 g/day has replaced the upper limit of 2.3 g/day.
[&] EAR magnesium 330 mg/day for men 19-30, 350 mg/day men aged 31 ->70

Appendix-II Additional information to support the nutrition assessment

Table A2.1. Estimated usual dietary intake of protein (g/day) for the Australian population (ABS 2015c)

Age group (years)	Mean intake from food	High intake from food (P95)	Estimated Average Requirement (EAR)	Proportion with usual intake less than EAR (%)
Males				
2-3	61	80	12	—
4-8	67	87	16	—
9-13	87	122	31	—
14-18	104	142	49	0.1
19-30	113	152	52	0.1
31-50	108	147	52	0.2
51-70	98	135	52	0.6
71 +	86	122	65	13.7
Females				
2-3	53	71	12	—
4-8	59	78	16	—
9-13	74	103	24	—
14-18	76	105	35	0.3
19-30	77	106	37	0.3
31-50	79	109	37	0.2
51-70	78	108	37	0.3
71 +	73	101	46	3.8

A dash '—' means zero or rounded to zero.

Table A2.2. Estimated usual dietary intake of protein (g/day) for the New Zealand population (MoH 2005; MoH 2011a; MoH 2011b)

Age group (years)	Mean intake	High intake (P90)	Estimated Average Requirement (EAR)*	Proportion with inadequate intake at baseline (%<EAR)[$]
Males				
5-6	63	81	16**	—
7-10	73	99	31***	—
11-14	91	125	49****	—
15-18	108	125	49****	0.0
19-30	113	140	52	0.0
31-50	113	142	52	0.0
51-70	92	124	52	1.7
71 +	79	95	65	13.4
Females				
5-6	54	72	16**	—
7-10	64	88	24***	—
11-14	67	87	35****	—
15-18	69	93	35****	0.7
19-30	73	97	37	0.5
31-50	79	103	37	0.3
51-70	71	95	37	1.6
71 +	62	83	46	15.5

[$] Proportion with inadequate intake at baseline for children up to 14 year based on UK dietary reference values (1991) and for adults from 15 years and older based on NRVs for Australia and New Zealand (2006).
*EARs for Australia and New Zealand, **EAR for 4-8 years, ***EAR for 9-13 years, ****EAR for 14-18 years
—No data available in 2002 NZ CNS (MoH 2005)

Table A2.3. Estimated usual dietary intake (at baseline) of selected dietary fats / fatty acids (mg/day) for the Australian population (ABS 2015c)

Age group (years)	Polyunsaturated fat		Saturated fat		Trans fatty acids		Linoleic acid			α-linolenic acid			Long-chain omega 3 fatty acids (DHA, EPA, DPA)				
	Mean intake	High intake (P95)	Mean intake	High intake (P95)	Mean intake	High intake (P95)	Mean intake	High intake (P95)	AI*	Mean intake	High intake (P95)	AI*	Mean intake	High intake (P95)	AI*	UL	Proportion with usual intake exceeding UL (%)
Males																	
2-3	6500	9000	23800	32900	1159	1697	5000	8000	5000	900	1300	500	96	170	40	3 000	—
4-8	8200	11200	26900	36700	1294	1884	7000	10000	8000	1000	1500	800	103	183	55	3 000	—
9-13	10700	17100	33700	51200	1616	2673	9000	15000	10000	1300	2200	1000	176	378	70	3 000	—
14-18	12200	19000	36400	54200	1865	3006	10000	16000	12000	1500	2400	1200	206	430	125	3 000	—
19-30	13600	21000	34100	51200	1691	2755	11000	18000	13000	1600	2600	1300	262	538	160	3 000	—
31-50	13000	20300	32800	49800	1659	2723	11000	17000	13000	1600	2600	1300	270	558	160	3 000	—
51-70	11700	18400	29100	45000	1483	2466	10000	15000	13000	1500	2400	1300	258	535	160	3 000	—
71 +	10100	16300	27400	43000	1493	2498	8000	13000	13000	1400	2300	1300	229	483	160	3 000	—
Females																	
2-3	5700	7900	20700	29100	983	1461	5000	7000	5000	800	1100	500	92	163	40	3 000	—
4-8	7200	9900	23500	32500	1101	1618	6000	8000	8000	900	1300	800	98	174	55	3 000	—
9-13	9900	15300	29400	44900	1411	2276	8000	13000	8000	1200	2000	800	162	297	70	3 000	—
14-18	10300	16100	28000	43400	1294	2124	9000	14000	8000	1200	2100	800	179	331	85	3 000	—
19-30	10500	16200	25000	38900	1175	1934	9000	14000	8000	1200	2100	800	187	340	90	3 000	—
31-50	10200	15700	24600	38400	1193	1961	8000	13000	8000	1200	2100	800	234	421	90	3 000	—
51-70	9900	15400	23000	36200	1142	1882	8000	13000	8000	1200	2100	800	241	432	90	3 000	—
71 +	8900	13800	22400	35200	1186	1940	7000	11000	8000	1200	2000	800	229	409	90	3 000	—

* AI = Adequate intake-the average daily nutrient intakes level based on observed or experimentally-derived approximations or estimated of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate (NHMRC and MOH 2017).

Note: no UL for linoleic acid and α-linolenic acid.
A dash '—' means zero or rounded to zero.

Table A2.4. Estimated usual dietary intake of polyunsaturated fat and saturated fat (mg/day) for the New Zealand population (MoH 2005; MoH 2011a; MoH 2011b)

Age group (years)	Polyunsaturated fat		Saturated fat	
	Mean intake at baseline	High intake at baseline (P90)	Mean intake at baseline	High intake at baseline (P90)
Males				
5-6	7700	9200	29100	33600
7-10	9000	11100	35800	43700
11-14	11000	17100	41800	58500
15-18	13300	17200	42500	50800
19-30	14300	22100	41700	53600
31-50	14800	19000	41200	53700
51-70	12300	18300	32900	51300
71 +	10400	14600	26500	38400
Females				
5-6	6700	9100	24900	31900
7-10	7900	10700	30500	41900
11-14	8700	11600	33700	47700
15-18	9200	12900	29100	40600
19-30	9900	14400	29600	45800
31-50	10500	13600	29200	36900
51-70	10300	15400	24600	37600
71 +	8000	10800	20400	29700

Table A2.5. Estimated usual dietary intake of selected vitamins (µg /day) for the Australian population (ABS 2015d)

Age group (years)	Cobalamin (Vitamin B_{12})				Dietary folate equivalents				Folic acid			
	Mean intake at baseline	High intake at baseline (P95)	EAR	Proportion with usual intake less than EAR (%)	Mean intake at baseline	High intake at baseline (P95)	EAR	Proportion with usual intake less than EAR (%)	Mean intake at baseline	High intake at baseline (P95)	UL	Proportion with usual intake greater than UL (%)
Males												
2-3	3.8	5.8	0.7	—	523	759	120	—	174	288	300	3.6
4-8	3.5	5.4	1	—	638	907	160	—	242	384	400	3.6
9-13	4.5	6.9	1.5	—	709	1105	250	0.4	255	458	600	0.7
14-18	5.4	8.1	2	0.1	752	1154	330	1.2	278	494	800	0.1
19-30	5.6	8.4	2	—	667	1041	320	2.8	220	425	1000	—
31-50	5.4	8.2	2	0.1	686	1071	320	2.3	225	421	1000	—
51-70	4.8	7.3	2	0.4	675	1057	320	2.5	219	406	1000	—
71 +	4.5	6.9	2	0.8	714	1115	320	1.6	248	442	1000	—
Females												
2-3	3.3	5.2	0.7	—	469	690	120	—	159	271	300	2.3
4-8	3.1	4.8	1	0.2	575	825	160	—	224	360	400	2.0
9-13	3.7	6.1	1.5	1.7	589	913	250	1.2	211	382	600	0.1
14-18	3.6	6	2	7.7	558	877	330	7.9	193	366	800	—
19-30	3.6	5.9	2	8.3	512	810	320	10.9	156	312	1000	—
31-50	3.9	6.3	2	5.5	517	818	320	10.6	154	307	1000	—
51-70	3.8	6.3	2	5.4	548	857	320	7.6	157	306	1000	—
71 +	3.8	6.2	2	5.8	565	877	320	6.1	177	329	1000	—

A dash '—' means zero or rounded to zero.

Table A2.6. Estimated usual dietary intake of selected vitamins (µg/day) for the New Zealand population (MoH 2005; MoH 2011a; MoH 2011b)

Age group (years)	Cobalamin (Vitamin B_{12})				Dietary folate equivalents			
	Mean intake	High intake (P90)	EAR*	Proportion with inadequate intake at baseline (%<EAR)[$]	Mean intake	High intake (P90)	EAR*	Inadequate intake %
Males								
5-6	3.4	4.8	1.0**	0	229	288	160**	0
7-10	3.8	5.3	1.5***	0	261	371	250***	0.9
11-14	4.5	7.2	2.0****	0.2	292	393	250***	0.6
15-18	4.6	6.5	2.0****	1.1	—	—	330****	—
19-30	5.3	6.6	2.0	0.0	—	—	320	—
31-50	5.2	7.3	2.0	0.2	—	—	320	—
51-70	5.2	8.7	2.0	2.8	—	—	320	—
71 +	5.7	10.2	2.0	3.8	—	—	320	—
Females								
5-6	2.6	3.6	1.0**	0	214	314	160**	0.2
7-10	3.1	4.3	1.5***	0	219	305	250***	1.7
11-14	3.4	4.9	2.0****	0	234	368	250***	20.2
15-18	3.7	5.5	2.0****	7.9	—	—	330****	—
19-30	3.6	6.3	2.0	22.8	—	—	320	—
31-50	3.7	6.2	2.0	16.1	—	—	320	—
51-70	3.5	4.6	2.0	1.1	—	—	320	—
71 +	3.2	5.4	2.0	27.0	—	—	320	—

[$] Proportion with inadequate intake at baseline for children up to 14 year based on UK dietary reference values (1991) and for adults from 15 years and older based on NRVs for Australia and New Zealand (2006).
*EARs for Australia and New Zealand, **EAR for 4-8 years, ***EAR for 9-13 years, ****EAR for 14-18 years
—No data available in 2008 NZ ANS (MoH 2011a; MoH 2011b)

Appendix-III Additional information on the dietary intake assessment

Table A3.1. Population groups assessed in the dietary intake assessments

Country	Survey	Population surveyed	Age groups assessed	Sex groups assessed
Australia	2011–12 NNPAS	2 years and above	2–3 years 4–8 years 9–13 years 14–18 years 19–30 years 31–50 years 51–70 years 71 years and over	Male and Female
New Zealand	2002 NZ CNS	5-14 years	5-6 years 7-10 years 11-14 years	Male and Female
	2008 NZ ANS	15 years and above	15–18 years 19–30 years 31–50 years 51–70 years 71 years and over	Male and Female

Table A3.2. Chicken meat consumption for Australia and New Zealand[#]

Country	Sex	Age group (years)	Mean consumption (g/day)	Consumption at P90 (g/day)
Australia*	Male	2-3	55	98
		4-8	61	113
		9-13	81	180
		14-18	111	182
		19-30	134	269
		31-50	112	228
		51-70	98	204
		>= 71	79	140
	Female	2-3	48	95
		4-8	50	101
		9-13	74	158
		14-18	91	186
		19-30	89	159
		31-50	89	179
		51-70	79	149
		>= 71	77	151
New Zealand^	Male	5-6	80	175
		7-10	83	192
		11-14	121	253
		15-18	159	369
		19-30	141	343
		31-50	163	326
		51-70	124	288
		>= 71	105	199
	Female	5-6	55	116
		7-10	84	169
		11-14	91	190
		15-18	106	206
		19-30	113	206
		31-50	117	243
		51-70	106	223
		>= 71	96	193

[#] As used for scenario 1: consumption amounts are representative of a longer term consumption pattern taking into account chicken meat that is eaten as a serve in its own right as well as other amounts from mixed dishes such as in main meals (e.g. stews or curries) or other uses such as in sandwiches.

* 2011-12 Australian National Nutrition and Physical Activity Survey. Based on consumption data from consumers with two days of data only.

^ 2002 New Zealand National Children's Nutrition Survey and the 2008–09 New Zealand Adult Nutrition Survey. Based on day 1 consumption data from consumers only.

Table A3.3. Estimated dietary intake of iron (mg/day) for the Australian population with additional contribution from the harvested cells[$]

Age group (years)	UL[*]	Mean usual intake at baseline [€]	High usual intake (P95) at baseline [€]	Mean intake from supplements [Σ]	Proportion with usual intake exceeding UL at baseline (%)[$]	Proportion with inadequate intake at baseline (% <EAR)[$]	Intake from the harvested cells for the scenario 1[£]		Intake from the harvested cells for the scenario 2[Ω]		Total intake for the scenario 1[¥]		Total intake for the scenario 2[¥]	
							Mean	High (P90)	Mean	High (P90)	Mean	High	Mean	High
Males														
2-3	20	8	12	3	—	8.5	0.331	0.588	0.85	1.80	11	16	12	17
4-8	40	9	13	3	—	5.9	0.366	0.676	0.85	1.80	12	17	13	18
9-13	40	12	17	4	—	3.3	0.488	1.083	0.85	1.80	16	22	17	23
14-18	45	13	19	4	—	8.3	0.668	1.095	0.85	1.80	18	24	18	25
19-30	45	13	19	8	—	2.2	0.804	1.616	0.85	1.80	22	29	22	29
31-50	45	13	19	7	—	2.2	0.673	1.368	0.85	1.80	21	27	21	28
51-70	45	12	18	4	—	2.8	0.589	1.222	0.85	1.80	17	23	17	24
71 +	45	12	18	6	—	3.1	0.473	0.838	0.85	1.80	18	25	19	26
Females														
2-3	20	7	10	3	—	14.8	0.291	0.568	0.85	1.80	10	14	11	15
4-8	40	8	12	3	—	10.8	0.300	0.606	0.85	1.80	11	16	12	17
9-13	40	9	14	5	—	10.5	0.447	0.948	0.85	1.80	14	20	15	21
14-18	45	9	14	10	—	40.1	0.546	1.119	0.85	1.80	20	25	20	26
19-30	45	9	14	12	—	37.5	0.531	0.951	0.85	1.80	22	27	22	28
31-50	45	9	14	8	—	37.5	0.535	1.077	0.85	1.80	18	23	18	24
51-70	45	10	15	5	—	5	0.477	0.892	0.85	1.80	15	21	16	22
71 +	45	9	14	6	—	6.7	0.464	0.905	0.85	1.80	15	21	16	22

[$]Iron concentration at 0.6 mg in 100 g of harvested cells was used.
*Upper Level for iron based on the Nutrient Reference Values for Australia and New Zealand (NHMRC and MoH 2006).
[€] Mean/ high usual intakes from food and beverages (ABS 2015e).
[Σ] Mean intake from supplements (ABS 2015b), day 1 data only.
[$] Proportion with usual intake exceeding UL and proportion with inadequate intake at baseline (ABS 2015e). A dash '—' means zero or rounded to zero.
[£] Consumers choose to eat the harvested cells at amounts equivalent to total longer term consumption of chicken meat, in addition to conventional meat including chicken.
[Ω] Consumers choose to consume the harvested cells at a proposed high serve size of 300 g/day (high consumption as suggested by Vow) or at a more average serve size of 142 g/day in addition to conventional meat including chicken.
[¥]Sum of mean/ high intake from food, dietary supplements and the harvested cells.

Table A3.4. Estimated dietary intake of iron (mg/day) for the New Zealand population with additional contribution from the harvested cells[$]

Age group (years)	UL[*]	Mean usual intake at baseline [€]	High usual intake at baseline (P90) [€]	Mean intake from supplements [Σ]	Proportion with inadequate intake at baseline (% <EAR)[$]	Intake from the harvested cells for the scenario 1[£]		Intake from the harvested cells for the scenario 2[Ω]		Total intake for the scenario 1[¥]		Total intake for the scenario 2[¥]	
						Mean	High (P90)	Mean	High (P90)	Mean	High	Mean	High
Males													
5-6	40	10	13	3	0	0.479	1.052	0.85	1.80	14	17	14	18
7-10	40	12	16	4	1.8	0.498	1.153	0.85	1.80	17	21	17	22
11-14	40-45**	14	19	4	2.1	0.728	1.516	0.85	1.80	19	24	19	25
15-18	45	14	19	4	5.0	0.952	2.211	0.85	1.80	19	25	19	25
19-30	45	14	20	8	0.1	0.849	2.056	0.85	1.80	23	30	23	30
31-50	45	14	20	7	0.8	0.978	1.955	0.85	1.80	22	29	22	28
51-70	45	13	19	4	1.3	0.742	1.729	0.85	1.80	18	25	18	25
71 +	45	12	16	6	1.3	0.629	1.193	0.85	1.80	18	23	19	24
Females													
5-6	40	9	11	3	0.1	0.328	0.698	0.85	1.80	12	14	12	16
7-10	40	10	13	5	5.0	0.505	1.017	0.85	1.80	15	19	16	19
11-14	40-45**	10	15	10	4.2	0.549	1.141	0.85	1.80	21	26	21	26
15-18	45	9	13	10	34.2	0.638	1.237	0.85	1.80	20	24	20	25
19-30	45	10	12	12	6.0	0.676	1.238	0.85	1.80	23	25	23	26
31-50	45	10	14	8	15.4	0.701	1.459	0.85	1.80	19	23	19	23
51-70	45	10	14	5	0.7	0.637	1.340	0.85	1.80	16	20	16	20
71 +	45	9	13	6	2.3	0.573	1.157	0.85	1.80	16	20	16	21

[$]Iron concentration at 0.6 mg in 100 g of harvested cells was used.

*Upper Level for iron based on the Nutrient Reference Values for Australia and New Zealand (NHMRC and MoH 2006).

**The UL for 9-13 years olds is 40 mg/day. The UL for 14 year olds is 45 mg/day.

[€] Mean and high usual intake from foods and beverages derived from the 2002 NZ CNS and 2008-09 NZ ANS (MoH 2003; University of Otago and MoH 2011). The highest reported usual intake in these publications is the 90th percentile; this has been used for the estimated high intakes from food for New Zealand.

[Σ] Australian mean intake for supplements for day 1 only was used (ABS 2015b). For 5-6 year olds the average used is for Australians aged 4-8 years. For 7-10 years olds the mean used is for Australians aged 9-13 years. For 11-14 year olds the average used is for Australians aged 14-18 years.

[$] Proportion with inadequate intake at baseline for children up to 14 year based on UK dietary reference values (1991) and for adults from 15 years and older based on NRVs for Australia and New Zealand (2006).

[£] Consumers choose to eat the harvested cells at amounts equivalent to total longer term consumption of chicken meat, in addition to conventional meat including chicken.

[Ω] Consumers choose to consume the harvested cells at a proposed high serve size of 300 g/day (high consumption as suggested by Vow) or at a more average serve size of 142 g/day in addition to conventional meat including chicken.

[¥]Sum of mean/ high usual intake from food and beverages (baseline), dietary supplements and the harvested cells. i.e. Mean = mean baseline intake + supplements + mean scenario intake; High = high baseline intake + supplements + high scenario intake.

Table A3.5. Estimated dietary intake of sodium (mg/day) for the Australian population with additional contribution from the harvested cells[$]

Age group (years)	UL/ SDT[*]	Mean usual intake at baseline [€]	High usual intake at baseline (P95) [€]	Proportion with usual intake exceeding UL at baseline (%)[$]	Intake from the harvested cells for the scenario 1[£]		Intake from the harvested cells for the scenario 2[Ω]		Total intake for the scenario 1[¥]		Total intake for the scenario 2[¥]	
					Mean	High (P90)	Mean	High (P90)	Mean	High	Mean	High
Males												
2-3	1000	1619	2127	99.2	65.54	116.33	168.55	356.10	1685	2243	1788	2483
4-8	1400	2158	2771	99.1	72.50	133.69	168.55	356.10	2230	2905	2327	3127
9-13	2000	2685	3959	83.2	96.58	214.17	168.55	356.10	2782	4173	2854	4315
14-18	2300	3146	4527	86.4	132.20	216.54	168.55	356.10	3278	4744	3315	4883
19-30	2000	3025	4368	82.7*	159.00	319.67	168.55	356.10	3184	4688	3194	4724
31-50	2000	2908	4235	78.7*	133.14	270.64	168.55	356.10	3041	4506	3077	4591
51-70	2000	2540	3756	60.7*	116.43	241.69	168.55	356.10	2656	3998	2709	4112
71 +	2000	2310	3474	46.6*	93.48	165.76	168.55	356.10	2403	3640	2479	3830
Females												
2-3	1000	1426	1898	95.3	57.49	112.37	168.55	356.10	1483	2010	1595	2254
4-8	1400	1924	2490	95.5	59.45	119.98	168.55	356.10	1983	2610	2093	2846
9-13	2000	2298	3258	68.7	88.39	187.47	168.55	356.10	2386	3445	2467	3614
14-18	2300	2357	3352	50.6	107.96	221.31	168.55	356.10	2465	3573	2526	3708
19-30	2000	2289	3247	46.1*	105.12	188.17	168.55	356.10	2394	3435	2458	3603
31-50	2000	2149	3072	35.9*	105.80	212.97	168.55	356.10	2255	3285	2318	3428
51-70	2000	1974	2844	24.0*	94.30	176.53	168.55	356.10	2068	3021	2143	3200
71 +	2000	1849	2675	16.5*	91.76	178.96	168.55	356.10	1941	2854	2018	3031

[$]Sodium concentration at 118.7 mg in 100 g of harvested cells was used.

*Upper Level for children up to 18 years, or Suggested Dietary Target for adults 19 years and over, for sodium based on the Nutrient Reference Values for Australia and New Zealand (NHMRC and MoH 2006).

[€] Mean/ high usual intakes from food and beverages (ABS 2015e).

[$] Proportion with usual intake exceeding UL (ABS 2015e).

** Compared with previously established UL of 2.3 g/day, which has since been replaced with a SDT of 2.0 g/day.

[£] Consumers choose to eat the harvested cells at amounts equivalent to total longer term consumption of chicken meat, in addition to conventional meat including chicken.

[Ω] Consumers choose to consume the harvested cells at a proposed high serve size of 300 g/day (high consumption as suggested by Vow) or at a more average serve size of 142 g/day in addition to conventional meat including chicken.

[¥]Sum of mean/ high usual intake from food and beverages, and the harvested cells. i.e. Mean = mean baseline intake + mean scenario intake; High = high baseline intake + high scenario intake.

Table A3.6. Estimated dietary intake of sodium (mg/day) for the New Zealand population with additional contribution from the harvested cells[$]

Age group (years)	UL/ SDT[*]	Mean usual intake at baseline [€]	High usual intake at baseline (P90) [€€]	Intake from the harvested cells for the scenario 1[£]		Intake from the harvested cells for the scenario 2[Ω]		Total intake for the scenario 1[¥]		Total intake for the scenario 2[¥]	
				Mean	High	Mean	High	Mean	High	Mean	High
Males											
5-6	1400**	1920	3840	94.76	208.02	168.55	356.10	2015	4048	2089	4196
7-10	2000***	—	—	98.43	228.10	168.55	356.10	—	—	—	—
11-14	2000****	2877	5754	144.05	299.95	168.55	356.10	3021	6054	3046	6110
15-18	2300****	—	—	188.41	437.44	168.55	356.10	—	—	—	—
19-30	2000[#]	2899	5798	167.88	406.66	168.55	356.10	3067	6205	3068	6154
31-50	2000[#]	2899	5798	193.39	386.78	168.55	356.10	3092	6185	3068	6154
51-70	2000[#]	2899	5798	146.85	342.01	168.55	356.10	3046	6140	3068	6154
71 +	2000[#]	2899	5798	124.43	236.02	168.55	356.10	3023	6034	3068	6154
Females											
5-6	1400**	1920	3840	64.98	138.07	168.55	356.10	1985	3978	2089	4196
7-10	2000***	—	—	99.89	201.12	168.55	356.10	—	—	—	—
11-14	2000****	2419	4838	108.52	225.76	168.55	356.10	2528	5064	2588	5194
15-18	2300[#]	—	—	126.27	244.80	168.55	356.10	—	—	—	—
19-30	2000[#]	2068	4136	133.68	244.97	168.55	356.10	2202	4381	2237	4492
31-50	2000[#]	2068	4136	138.72	288.73	168.55	356.10	2207	4425	2237	4492
51-70	2000[#]	2068	4136	126.00	265.02	168.55	356.10	2194	4401	2237	4492
71 +	2000[#]	2068	4136	113.45	228.98	168.55	356.10	2181	4365	2237	4492

[$]Sodium concentration at 118.7 mg in 100 g of harvested cells was used.

*Upper Level for children up to 18 years, or Suggested Dietary Target for adults 19 years and over, for sodium based on the Nutrient Reference Values for Australia and New Zealand (NHMRC and MoH 2006).

UL for age group 4-8, ***UL for age group 9-13** UL for age group 14-18

[#] SDT for adults 19 years and over

[€] Mid-bound value was used as the mean baseline usual intake from food and beverages extracted from New Zealand total diet study.

[€€] High baseline usual intake (90P) was considered as double the mean baseline usual intake (FSANZ 2009b)

[£] Consumers choose to eat the harvested cells at amounts equivalent to total longer term consumption of chicken meat, in addition to conventional meat including chicken.

[Ω] Consumers choose to consume the harvested cells at a proposed high serve size of 300 g/day (high consumption as suggested by Vow) or at a more average serve size of 142 g/day in addition to conventional meat including chicken.

[¥]Sum of mean/ high intake from food and the harvested cells. i.e. Mean = mean baseline intake + mean scenario intake; High = high baseline intake + high scenario intake.

— No data available for the age/sex group.

Appendix-IV Microbiological hazard identification

Microbiological hazard identification[1]

Table A4.1. Cell sourcing and cell banking[2]

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
1.	Cell sourcing (Embryonic fibroblasts from eggs)	Pathogens (bacteria, viruses, fungi, parasites, protozoa, mycoplasma)	Pathogens may be present in the cells and eventually carried to the end product where they could be pathogenic. Vertical transmission and associated with reproductive organs in hens	Testing of the cell line for presence of specific pathogens before cell banking Sourcing from healthy animals. Sourcing animals produced under GAP Access to animal records, information on animal health, housing etc	Test results for specific avian pathogenic bacteria, viruses, mycoplasma Veterinary Certification supplied verifying official monitoring on farm and of eggs
		Prions	Avian prion proteins are not a risk to humans as structurally different.	NR	NR
		Microbial toxins	Pathogens that can produce heat-stable toxins may be present in the cells.	Testing of the cell line for presence of specific toxin producing pathogens before cell banking	Not assessed
		AMR bacteria	Pathogens may be present in the cells and eventually carried to the end product where they could be pathogenic.	Sourcing from healthy animals. Sourcing animals produced under GAP Testing of the cell line for presence of specific pathogens before cell banking	Not assessed
2.	Cell culturing/ banking (Cell line developer)	Pathogens (bacteria, viruses, fungi, parasites, protozoa, mycoplasma) and pathogenic agents	Pathogens present in the cell culture media components or other reagents may be present in end product where they could be pathogenic	Raw material quality control programme Avoid use of animal-derived components Follow good practice guidelines (e.g. GCCP)	Cell line developer declaration that cell line is free from viruses, bacteria, including *Mycoplasma* spp. Sterility testing performed as per European Pharmacopeia

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
				Storage of cell banks below -80°C	guidelines. Data provided
		Pathogens (bacteria, viruses, fungi, mycoplasma)	Contamination due to unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or consumed	Follow good practice guidelines (e.g. GCCP), Aseptic handling of cells and inputs	Sterility testing performed as per European Pharmacopeia guidelines. Data provided
		AMR bacteria	Antibiotics and fungicides used in primary cell line isolation	Antibiotics and fungicides not used during establishment of master or working cell bank	Not tested or data not supplied
		Microbial endotoxins/ other microbial compounds	Heat stable LPS contaminating equipment and present in cell culture – other sources could be media and media components and recombinant proteins made in *E. coli*	Testing for the presence of Enterobacteriaceae and *E. coli*	Not specifically tested for but would be covered by sterility testing
		Cross contamination between cell lines of different origins or species	Unexpected presence of pathogens or pathogenic agents (e.g. prions) originating from the contaminating cell line	Follow good practice guidelines (e.g. GCCP, GHP)	Not tested or data not supplied. Indirectly covered by cell line analysis
3.	Cell culturing/ banking (Vow)	Pathogens (bacteria, viruses, fungi, parasites, protozoa, mycoplasma) and pathogenic agents	Pathogens present in the cell culture media components or other reagents may be present in end product where they could be pathogenic	Raw material quality control programme Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP) Storage of cell banks below -80°C	Test results for master cell bank (Endogenous retroviruses, *Chlamydophila* spp., Influenza Type A, Newcastle Disease) Test results for working cell bank (*Mycoplasma* spp.) Storage conditions not assessed
		Pathogens (bacteria, viruses, fungi, mycoplasma)	Contamination due to unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or	Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP) Training and monitoring	Not assessed

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
			consumed e.g. faecal coliforms, *E. coli* and *Salmonella* spp.		
		Microbial endotoxins	Heat stable LPS can contaminate equipment and become present in cell culture – other sources could be from media and media components and recombinant proteins made in *E. coli*	Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP)	Not assessed
		Cross contamination between cell lines of different origins or species	Unexpected presence of pathogens or pathogenic agents originating from the contaminating cell line	Follow good practice guidelines (e.g. GCCP, GHP)	Indirectly covered by cell line analysis

[1]Specific steps that were assessed. [2]Based on FAO/WHO (2023). NR = not required. Not assessed = covers potential hazards through the process considered by FSANZ as part of the assessment procedure but are either not required for this application or data was not provided and no assessment could be performed.

Table A4.2. Cell expansion / Production of cultured cells[1,2]

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
1.	Cell expansion (Shake flasks)	Pathogens (bacteria, viruses, fungi, parasites, protozoa, Mycoplasma)	Pathogens present in the cell culture media components or other reagents may be present in end product where they could be pathogenic	Raw material quality control programme Process monitoring Aseptic handling Visual monitoring / standard microbial analyses for microbial contamination Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP)	Not assessed
		Pathogens (bacteria, viruses, fungi, Mycoplasma)	Contamination due to unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or consumed e.g. faecal coliforms, *E. coli* and *Salmonella* spp.	Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP) Training and monitoring Environmental monitoring	Not assessed
		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; and if toxins are present in the final product they may lead to foodborne disease	Raw material quality control programme Aseptic handling Proposed visual monitoring for microbial contamination – no data provided	Not assessed
		Cross contamination between cell lines of different origins or species	Unexpected presence of pathogens or pathogenic agents (e.g. prions) originating from the contaminating cell line	Follow good practice guidelines (e.g. GCCP, GHP) Training, monitoring and record keeping	Not assessed
2.	Cell expansion	Pathogens (bacteria, fungi,	Pathogens present in the cell culture media components or	Raw material quality control programme	No monitoring data provided, assessed harvested cells data

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
	(Bioreactor)	mycoplasma)	other reagents may be present in end product where they could be pathogenic	Process monitoring Aseptic handling Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP)	as proxy for in process monitoring
		Pathogens (bacteria, fungi, mycoplasma)	Additional scale up of process to larger volumes may increase difficulty in maintaining sterility	Raw material quality control programme Process monitoring Aseptic handling	Not assessed beyond scale in application
		Pathogens (bacteria, fungi, mycoplasma)	Build-up of pathogenic bacteria in the equipment overtime (e.g. biofilms) that may be present in end product where they could be pathogenic	Pre-Operation checks (e.g. ATP swabs, standard microbial analyses) Monitoring and recording of chemical cleaners and sanitisers parameters (e.g. pH, time, temperature and concentration)	Not assessed
		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; and if toxins are present in the final product they may lead to foodborne disease	Raw material quality control programme Aseptic handling Visual monitoring / standard microbial analyses for microbial contamination	Not assessed
3.	Cell expansion (Draw and fill)	Pathogens (bacteria, fungi, mycoplasma)	Pathogens present in the cell culture media components or other reagents may be present in end product where they could be pathogenic.	Raw material quality control programme Process monitoring Aseptic handling Follow good practice guidelines (e.g. HACCP + GCCP, GHP, GMP) Training and monitoring	Not assessed
		Pathogens	Contamination due to	Follow good practice guidelines (e.g.	Not assessed

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
		(bacteria, viruses, fungi, mycoplasma)	unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or consumed	HACCP + GCCP, GHP, GMP) Training and monitoring Environmental monitoring	
		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; and if toxins are present in the final product they may lead to foodborne disease	Raw material quality control programme Aseptic handling Visual monitoring / standard microbial analyses for microbial contamination	Not assessed

[1]Specific steps that were assessed. [2]Based on FAO/WHO (2023). Not assessed = covers potential hazards through the process considered by FSANZ as part of the assessment procedure but are either not required for this application or data was not provided and no assessment could be performed.

Table A4.3. Harvested cells including harvesting of cultured cells[1,2]

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
1.	Centrifugation	Pathogens (bacteria, fungi, mycoplasma)	Build-up of pathogenic bacteria in equipment overtime (e.g. biofilms of filters, seals and joints) that may be present in end product where they could be pathogenic e.g. *Listeria monocytogenes*,	Pre-Operation checks (e.g. ATP swabs, standard microbial analyses) Monitoring and recording of chemical cleaners and sanitisers parameters (e.g. pH, time, temperature and concentration)	Not assessed
		Pathogens (bacteria, fungi, mycoplasma)	Contamination due to unhygienic operators, environment or equipment could be carried to the end product and be hazardous when handled or consumed e.g. faecal coliforms, *E. coli* and *Salmonella* spp.	Process monitoring Aseptic handling Follow good practice guidelines (e.g. HACCP, GHP, GMP) Training and monitoring	Assessed microbiological data on consecutive product batches (n=5); following one cycle freeze/thaw
		Pathogens (bacteria, fungi, mycoplasma) and pathogenic agents (e.g. prions)	Pathogens present in washing or reagents may be present in end product where they could be pathogenic.	Raw material quality control programme Avoid unnecessary handling and washing of cell biomass Aseptic handling Follow good practice guidelines (e.g. HACCP, GHP, GMP)	Not assessed
		Pathogens – bacteria, viruses, fungi,	Cross contamination between different batches of harvested cells could lead to spread of bacteria/fungi/viruses that may be present in end product where they could be pathogenic	Process monitoring Follow good practice guidelines (e.g. HACCP, GHP, GMP)	Not assessed

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
		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; and if toxins are present in the final product they may lead to foodborne disease	Raw material quality control programme Process monitoring Aseptic handling Follow good practice guidelines (e.g. HACCP, GHP, GMP) Training and monitoring	Not Assessed
2.	Collection (vessels)	Pathogenic contaminants (bacteria, viruses, fungi, parasites)	Pathogenic contaminants due to unhygienic operators, equipment or environment could be carried to the end product and be hazardous when handled or consumed e.g. *Listeria monocytogenes; Salmonella* spp.	Temperature control to limit bacterial growth Aseptic handling Follow good practice guidelines (e.g. HACCP, GHP, GMP) Training and monitoring	Videos of cell mass collection as supplied No microbiological data assessed
		Pathogens (bacteria, fungi, mycoplasma) and pathogenic agents (e.g. prions)	Cross contamination between different batches of harvested cells	Follow good practice guidelines (e.g. HACCP, GHP, GMP) Process monitoring	Not assessed
3.	Cell biomass packaging and freezing	Pathogens – bacteria, viruses, fungi, parasites, protozoa	Pathogenic contaminants due to unhygienic operators, equipment or environment could be carried to the end product and be hazardous when handled or consumed e.g. *Listeria monocytogenes; Salmonella* spp	Temperature controls to limit bacterial growth Aseptic handling Follow good practice guidelines (e.g. HACCP, GHP, GMP) Training and monitoring	Assessed product cooling/freezing profiles, at current scale of production Assessed microbiological data on consecutive product batches (n=5); following one cycle freeze/thaw Assessed microbial stability data (*Listeria)* during refrigerated storage of cell mass over time.

[1]Specific steps that were assessed. [2]Based on FAO/WHO (2023). Not assessed = covers potential hazards through the process considered by FSANZ as part of the assessment procedure but are either not required for this application or data was not provided and no assessment could be performed.

Table A4.4. Harvested cells and the final food product[1,2]

	Production step	Hazard ID	Description and other relevant information	Expected mitigation	Information provided and assessment status
1.	Thawing/freezing cell mass	Pathogenic contaminants (bacteria, fungi)	Pathogenic contaminants due to unhygienic operators, equipment or environment could be carried to the end product and be hazardous when handled or consumed e.g. *Listeria monocytogenes; Salmonella* spp.	Temperature control to limit bacterial growth Aseptic handling of cell mass Follow good practice guidelines (e.g. GHP)	Process not assessed as final food product was not part of this application
2.	Addition of ingredients to form food	Pathogens (bacteria, fungi, mycoplasma) and pathogenic agents (e.g. prions, toxins)	Pathogenic contaminants present in ingredients which are added to the cell mass, could be carried to the end product and be hazardous when handled or consumed	Raw material quality control programme Aseptic handling of cell mass Cleaning and sanitising of equipment etc Product testing	Not assessed as final food product was not part of this application
3.	Final product	Pathogens (bacteria, fungi, mycoplasma) and pathogenic agents (e.g. prions, toxins)	Pathogenic contaminants present in final food and be hazardous when handled or consumed	Preparation in a manner which manages the hazard before consumption e.g. Heating/cooking, to an appropriate temperature/time	Not assessed as final food product was not part of this application

[1]Specific steps that were assessed. [2]Based on FAO/WHO (2023). Not assessed = covers potential hazards through the process considered by FSANZ as part of the assessment procedure but are either not required for this application or data was not provided and no assessment could be performed.

Appendix-V Summary of clarifications to SD1

Summary of the main clarifications made to the risk assessment following the 1st CFS are:

- Modifications to the conclusions section of the Executive Summary.
- Revisions to Sections 2.2.1, 2.2.1.2 and 3.3.1 in line with comments received from DAFF.
- Minor editorial changes to Section 3.1 to reflect for the basal media inputs, consideration was given to the identity, source, and any hazards associated with these substances. This included consideration of toxicological information and/or risk assessments of the substances by overseas agencies.
- The footnote to Section 3.1 was amended to clarify that substances are being used to support the growth of the cells during culture and are not performing a technical function either during food processing, or in the final food. Nor are they added with the intent of being an ingredient in the final food.
- Section 4.2.3.1 was revised to include a new footnote.
- Section 4.4.1 – FSANZ identified additional microorganisms to be incorporated into their process control monitoring and a pathogen to be included in product monitoring. The *Compendium of Microbiological Criteria for Food*[23] will be updated to include relevant microbial indicator organisms for monitoring the performance of the process along with sampling and analysis guidance.
- Section 4.4.2 was revised to include further details regarding the specification for protein. Changes have also been made to Table 6.
- Section 4.5 was revised to include microbiological criteria for indicator organisms and pathogen micro-limits for food safety.
- Section 5.1 was revised providing more information on scale of production, microbial hazards and risk management.
- Section 5.1.5 was revised to align with revisions in noted sections above.

[23] https://www.foodstandards.gov.au/publications/Compendium-of-Microbiological-Criteria-for-Food