Vow Cultured Quail

Novel Food Dossier

Food Standards Australia New Zealand

UPDATE

Submitted by:
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GLOSSARY OF ACRONYMS

ADI	Acceptable Daily Intake
AI	Adequate Intake
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
BMV	Brome mosaic virus
BOLD	Barcode of Life Data System informatics workbench
Bw	Body weight
CODEX	Codex Alimentarius collection of food standards
CFU	Colony forming unit
COI	Cytochrome C oxidase sub-unit 1
csMCB	Cell-line Supplier Master Cell Bank
csWCB	Cell-line Supplier Working Cell Bank
DNA	Deoxyribonucleic acid
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunoassay
EU	European Union
FAO	Food and Agriculture Organization
FDA	United States Food and Drug Administration
F-PERT	Fluorescent Product Enhanced Reverse Transcriptase
FSANZ	Food Standards Australia New Zealand
GCCP	Good Cell Culturing Practice
GLP	Good Lab Practice
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HEPA	High-Efficiency Particulate Absorbing Filter
HPB	Health Promotion Board
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IgE	Immunoglobulin E
JEFCA	Joint FAO/WHO Committee on Food Additives
UPLC-ESI-MS/MS	Ultrahigh Performance Liquid Chromatography - Electrospray Ionisation Ion Trap tandem Mass Spectrometry
LOAEL	Lowest Observable Adverse Effects Level
LOF	Loss of Function
MEXT	Ministry of Education, Culture, Sports, Science and Technology
MOE	Margin of Exposure
NOAEL	No Observable Adverse Effects Level
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
qPCR	Quantitative Polymerase Chain Reaction

RDA	Recommended Daily Allowance
RDI	Recommended Dietary Intake
RNA	Ribonucleic acid
RT	Reverse Transcriptase
SCGM	Suspension Cell Growth Medium
SDT	Suggested Dietary Target
SFA	Singapore Food Agency
SNV	Single Nucleotide Variants
SOP	Standard Operating Procedure
STR	Stirred Tank Reactor
TCS	Trypto-Casein-Soy
THIO	Thioglycolate
TTC	Threshold of Toxicological Concern
UL	Upper Limit
ULA	Ultra Low Attachment
US	United States
USDA	United States Department of Agriculture
vMCB	Vow Master Cell Bank
vWCB	Vow Working Cell Bank
WGS	Whole Genome Sequencing
WHO	World Health Organization

GENERAL REQUIREMENTS

This section is completed in accordance with Chapter 3.1 (General Requirements for Applications) of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2019).

1. Form of the application

1.1 Language

The application is in English.

2. Applicant details

2.1 Applicant (individual or organisation's) name and address

Vow Group Pty Ltd
6 Ralph St
Alexandria NSW 2015
Australia

2.2 Name of contact person

Vow Contact

[Personal details of Vow contact have been kept confidential for privacy reasons]

2.3 Nature of applicant's business

Vow Group Pty Ltd ("Vow") is a biotechnology company that uses cell culturing techniques to grow meat externally from animals. The company was founded in 2019 in Sydney, Australia and has since been working on developing and refining its first product - cultured quail cells. Vow's cultured quail cells are a novel ingredient that will be used initially as the main source of protein in high quality restaurant style dishes. The product is intentionally versatile so that Vow's partners have the flexibility to create unique and original culinary experiences for consumers.

2.4 Details of other individuals, companies or organisations associated with the application.

Agent Contact

[Personal details of Agent have been kept confidential for privacy reasons]

3. Purpose of the application

Vow is submitting this application to amend the Australia New Zealand Food Standards Code ("Food Standards Code") to allow the use of Vow cultured quail made from the isolated embryonic fibroblasts of *Coturnix japonica*, or Japanese quail, as a component in food products to be marketed and sold in Australia and New Zealand. Vow cultured quail may be mixed with other authorised, food-grade ingredients to form products such as logs, rolls, and patties.

Vow cultured quail is considered to be a novel food according to the definition in Standard 1.5.1 of the Food Standards Code, given "the process by which the food has been prepared" and "the source from which it is derived." Although there is a long history of consumption of quail, specifically Japanese quail, in Australia and New Zealand, Vow's cultured quail is created *in vitro* using novel cell culturing processes. Vow cultured quail is similar to conventional quail in composition (see Section C.4 for more information from comparison testing to conventional quail meat).

It is the view of Vow that the following Schedules will need to be amended to allow Vow cultured quail to be used as a food ingredient in products marketed and sold in Australia and New Zealand:

Schedule 3: Identity and purity
Schedule 22: Foods and classes of foods
Schedule 25: Permitted novel foods

4. Justification for the application

The human population is projected to grow to 9.7 billion by 2050 (UN, 2022). Rising incomes along with rising population will increase global demand for protein by over 40%. As leading global exporters of protein, Australia and New Zealand will need to develop new technologies to help meet the domestic and international increases in demand. In addition, animal disease and climate change threaten existing sources of protein. Cultured meat can help to support the growing demand for protein and make the food industry more resilient. By 2030, there is a $25 billion opportunity for the cultured meat industry globally (McKinsey & Company, 2021). Australia and New Zealand have an opportunity to seize on this opportunity by leading in the regulatory space, ensuring that cultured meat is safe and ready for consumption.

No cultured meat products are currently available for sale in Australia and New Zealand. Vow is submitting this application to seek approval for its first cultured meat ingredient to be used in food products. Approving the ingredient can provide an advantage to Australia and New Zealand, helping to establish the countries as leaders in further development of this technology. The approval will also help FSANZ to establish an approach for evaluating future cultured food applications, helping to make it a global leader among food regulators in this space. Consumers will have the advantage of having more protein choices, alongside existing livestock meat and plant-based proteins.

At the time of submission, Vow has also sent an application to the Singapore Food Agency for review and subsequent approval pending the outcome of their risk assessment.

4.1 Regulatory impact information

4.1.1 Costs and benefits considerations of the application

(a) Key considerations for consumers:

- **Minimising the development of antimicrobial resistance -** Vow's cultured quail meat is produced in antibiotic free media which means that our final product does not contribute to the growing presence of antimicrobial resistance.
- **Price -** There is no direct impact on the price of traditional protein sources. Although Vow's cultured quail product will likely be more expensive, initially consumers will have the choice to select a product suitable for them.

(b) *Key considerations for industry:*

- **Job creation and/or upskilling.** The production of Vow's cultured quail meat will require the creation and ongoing day-to-day running of domestic manufacturing facilities to produce Vow's product. These facilities will create jobs for both highly skilled (*i.e.*,

scientists, process engineers, trades people) and unskilled workers (*i.e.*, manufacturing technicians, cleaning, rubbish removal).
- **Increased business for restaurateurs and small businesses.** Vow's restaurant partners are likely to benefit from the increased consumer interest and demand for our product.

(c) Key considerations for government:

In November 2022, the Food Ministers' Meeting, composed of Ministers from Australian states and territories and the Australian and New Zealand federal governments, affirmed the view of FSANZ that the existing novel foods pre-market approval process is fit-for-purpose for new cultured meat products (Australian Government Department of Health, 2022).

This proposed change places no additional regulatory cost on the government beyond the initial cost associated with reviewing the amendment submission and the cost of enforcing the relevant manufacturing and food safety regulations.

4.1.2 Impact on international trade

Currently, Singapore has approved the commercial sale of a cultured meat product; the US FDA has completed two pre-market consultations for cultured meat products which have been reviewed by USDA. This means that the international trade of such goods is limited and not yet established.

Vow is seeking approval to sell a distinct product (cultured quail) across multiple jurisdictions (*e.g.* Singapore, Australia and New Zealand).

Therefore, this proposed change will positively affect international trade by placing Australia/New Zealand in a unique position to increase its market share through the export of cultured quail to other markets. Australia/New Zealand will be seen as a leader in this growing industry by signalling to other markets and will significantly contribute to reducing technical barriers to trade.

5. Information to support the application

5.1 Data requirements

To identify publications relevant to the safety of cultured quail, a comprehensive search of the published scientific literature was conducted for any publications up to 4 January 2023 using the databases CINAHL, FSTA®, MEDLINE®, Proquest Environmental Science Index, and Toxline. All information in the application is obtained, described, and referenced as indicated in Section E.1 Data Requirements of the FSANZ Application Handbook (2019), with copies provided to FSANZ.

As detailed in Section C.3, no published studies were identified that suggested allergenic, toxic, or adverse health effects related to the consumption of cultured quail.

6. Assessment procedure

Vow considers the General Procedure (Subdivision D of the FSANZ Act) to be the most appropriate assessment procedure for this application. Vow believes the application falls under the Level 4 classification.

This is due to the history of consumption of quail meat in Australia and New Zealand, and the similarity of Vow cultured quail meat to it, recognising the additional attention in assessing an early application using cell culturing techniques.

7. Confidential commercial information (CCI)

Confidential commercial information, in relation to food, is defined in Subsection 4(1) of the FSANZ Act as meaning:

1. A trade secret relating to food; or
2. Any other information relating to food that has a commercial value that would be, or could reasonably be expected to be, destroyed or diminished if the information were disclosed.

Vow requests the information contained within the following appendices be considered confidential commercial information (CCI):

- Appendix 3 Mycoplasma Reports [CCI]
- Appendix 4 Sterility Report [CCI]
- Appendix 5 Retrovirus Report [CCI]
- Appendix 6 Species Specific Bacteria Report [CCI]
- Appendix 7 Species Specific Virus Report [CCI]
- Appendix 8 Microbiological Reports [CCI]
- Appendix 9 Heavy Metal Reports [CCI]
- Appendix 10 Antibiotic Reports [CCI]

Appendices 3-10 provide confidential details on Vow's cell supplier and their cell line. Non-confidential descriptions of these appendices are provided in section B.4.

- Appendix 11 Manufacturing Process [CCI]
- Appendix 12 Veterinary Certificate [CCI]
- Appendix 13 HACCP Plan [CCI]
- Appendix 14 GCCP Plan [CCI]

Appendices 11-14 provide confidential details pertaining to Vow's manufacturing process. Non-confidential descriptions of these appendices are provided in section B.5.

- Appendix 15 Species Confirmation [CCI]

Appendix 15 provides confidential details on Vow's cell supplier and their cell line. A non-confidential description of this appendix is provided in section C.6.1.2.

- Appendix 18 Allergen Reports [CCI]
- Appendix 19 Laboratory Accreditations [CCI]
- Appendix 20 Gross Composition and Nutrient Testing Methods [CCI]
- Appendix 21 Proximate Reports [CCI]
- Appendix 23 Amino Acid Statistics [CCI]

Appendices 18-23 provide confidential details about the components of Vow's cultured quail product. Non-confidential descriptions of these appendices are provided in sections C.6.1.4 and C.6.2.

- Appendix 24 Reagent CoAs [CCI]
- Appendix 25 Reagent and Processing Aid Risk Assessment [CCI] [CCI]
- Appendix 26 Residue Measurements [CCI]
- Appendix 27 Growth Factor CoAs [CCI]
- Appendix 28 Growth Factor Reports [CCI]
- Appendix 29 Genetic Stability [CCI]

Appendices 24-29 provide confidential details on the components used in the media. Non-confidential descriptions of these appendices are provided in section C.6.3.

- Supplemental Appendix 1 Microbiological Results [CCI]
- Supplemental Appendix 2 Proximate Reports [CCI]
- Supplemental Appendix 3 Amino Acid Stats [CCI]
- Supplemental Appendix 4 Residue Measurements [CCI]
- Supplemental Appendix 5 Reagent and Processing Aid Risk Assessment [CCI]
- Supplemental Appendix 6 Growth Factor #2b CoA and Background Reports [CCI]
- Supplemental Appendix 7 Growth Factor Results [CCI]
- Supplemental Appendix 8 Allergen Reports [CCI]
- Supplemental Appendix 9 Bioinformatic Reports [CCI]

Supplemental Appendices 1-9 provide confidential details on the testing and analyses conducted on Vow cultured quail manufactured with Growth Factor #2b. Non-confidential descriptions of these appendices are provided in the Supplement, "FSANZ Supplemental Update".

8. Other confidential information

No other confidential information is included in this application.

9. Exclusive capturable commercial benefit (ECCB)

Given that Vow cultured quail is produced using a proprietary cell culturing process, it is anticipated that this application would confer Exclusive Capturable Commercial Benefit (ECCB), which according to Section 8 of the FSANZ Act is conferred when:

a) the applicant can be identified as a person or body that may derive a financial gain from the coming into effect of the draft standard or draft variation of the standard that would be prepared in relation to the application; and

b) any other unrelated persons or bodies, including unrelated commercial entities, would require the agreement of the applicant in order to benefit financially from the approval of the application.

10. International and other national standards

10.1 International Standards

There are no Codex Alimentarius Commission standards relevant to Vow's cultured quail.

10.2 Other national standards or regulations

At the time of submission there are no other national standards or regulations related to Vow's cultured quail.

11. Statutory declaration

A signed Statutory Declaration will be provided for final submission in Appendix 1.

12. Checklist

A complete checklist is provided in Appendix 2.

NOVEL FOODS

This section is completed in accordance with Section A of Guideline 3.5.2 – Novel foods – of the *Food Standards Australia New Zealand Application Handbook* (FSANZ, 2019).

A. Exclusive use of novel foods

Vow is not seeking exclusive permission to market its cultured quail should it be approved by FSANZ as a novel food.

B. Technical information on the novel food

Technical information on the cultured quail produced by Vow is described in this section. The cultured quail evaluated in this dossier is representative of the commercial product for which approval is sought. This section is completed in accordance with the information requirements in relevant sections of Guideline 3.5.2. (Novel Foods) of the *Food Standards Australia New Zealand Application Handbook (2019)*.

B.1 Information on the type of novel food

Vow's cultured quail product is an ingredient manufactured from the isolated embryonic fibroblasts of *Coturnix japonica*, or Japanese quail. The product consists of cultured quail fibroblasts and is similar in composition to conventional *C. japonica* quail muscle. The ingredient has a protein content of > 4 %, a moisture content of > 80 %, and a fat content of 0.5-3 %. Vow cultured quail is currently manufactured in a factory designed for low contamination risk, following Standard Operating Procedures. Vow has implemented a Hazard Analysis and Critical Control Point (HACCP) food safety management system to control potential food safety hazards. Commercial production is in compliance with Good Cell Culture Practice (GCCP). Vow cultured quail is manufactured using inputs evaluated for suitability as food ingredients and processing aids and is intended for use as an ingredient in food.

Vow cultured quail falls within the following major novel categories listed in Section 3.5.2. (Novel Foods):

1. Foods derived from new sources
2. Foods produced by a process not previously applied to food

B.2 Information on the purpose of adding a novel food ingredient to food

Vow is seeking to allow the use of cultured quail as an ingredient in food products sold in Australia and New Zealand. Vow cultured quail is manufactured using a novel process to grow cells outside of animals. The cells grow within bioreactors but become non-viable and stop growing shortly after harvest. Similar to conventional meat, the cells are not viable when served to the consumer. The cultured quail may be mixed with other authorised, food-grade ingredients to produce formed products such as logs, rolls, patties, etc., for use at a maximum

300 g of Vow cultured quail per portion, to be cooked and served in restaurants by food service professionals.

B.3 Information on the physical and chemical properties of the novel food or novel food ingredient

Vow cultured quail is derived from the embryonic fibroblasts of Japanese quail (*Coturnix japonica*). Fibroblasts are the main active cells of connective tissue. Full details on composition are described in Sections C.6.1.1 (Identity), and C.6.2 (Information on the composition of the novel food ingredient derived from a new source).

B.4 Information on the impurity profile for a typical preparation

As with all food preparations, the microbiological and chemical impurities were evaluated. Microbiological analysis included testing for mycoplasma, sterility, retrovirus, species specific bacteria, species specific viruses, and final product analysis. Testing for chemical impurities include heavy metals and antibiotics.

B.4.1 MICROBIOLOGICAL ANALYSIS

Microbiological testing prioritised the detection of viruses, bacteria and other microbes that:

1. Are poultry/quail pathogens and/or common cell culture contaminants;
2. Have zoonotic potential; and,
3. Pose a food-safety concern if consumed in meat.

Common cell culture contaminants and poultry pathogens were identified during development of the food safety plan. An emphasis was placed on microorganisms that can be introduced in traditional food manufacturing, into cell culture in an R&D setting, during pharmaceutical manufacturing, and/or be present in conventional quail livestock. Zoonotic potential was assessed via pathogen host-specificity, examples of human infections, and/or the presence of pathogen-specific antibodies following exposure. The microbes tested represent quail pathogens with zoonotic potential through oral routes (*i.e.,* consumption). If testing indicated positive results for any of the listed adventitious agents, the cells would be immediately destroyed and discarded.

Table B.4.1-1 details Vow's adventitious testing strategy, including test method and a description of the sample that is tested. The cell-line supplier Master Cell Bank (csMCB) is the cell line sourced from a cell-line supplier. The Vow Master Cell Bank (vMCB) is the Master cell bank produced by Vow from the csMCB. The Vow Working Cell Bank (vWCB) is derived from the vMCB, used to create the seed train for cell expansion. The harvested cells are the final cultured quail product, harvested from the bioreactor and intended to be used as an ingredient in food.

Table B.4.1-1: Summary of adventitious agent testing

Parameter tested	Method	Processing stage	Result
Mycoplasma	Quantitative polymerase chain reaction (qPCR)	csMCB	Negative
	Nucleic acid based detection	vWCB	Negative
Sterility	Direct inoculation and microbial cell growth	csMCB	Negative
Endogenous retrovirus	Real Time Fluorescent Product Enhanced Reverse Transcriptase (F-PERT) assay	vMCB	Negative
Species specific bacteria – *Chlamydophila spp.*	PCR	vMCB	Negative
Species specific viruses – Influenza type A, Newcastle Disease	PCR	vMCB	Negative
Standard plate count	Pour plate	Harvested cells	$< 10^4$ CFU/g
Coliforms	Spread plate - Petrifilm	Harvested cells	< 100 CFU/g
E. coli	Most probable number	Harvested cells	< 3 MPN/g
Enterobacteriaceae	Spread plate - Petrifilm	Harvested cells	< 100 CFU/g
Salmonella	Enzyme linked immunosorbent assay (ELISA) – SOLUS	Harvested cells	Not detected in 25 g
Listeria monocytogenes	Enzyme linked immunosorbent assay (ELISA) – SOLUS	Harvested cells	Not detected in 25 g
Coagulase positive *Staphylococcus aureus*	Spread plate	Harvested cells	$< 10^2$ CFU/g

B.4.1.1 Mycoplasma

In cell culture, mycoplasma contamination of cell cultures can occur from animal-derived media components and from the introduction of mycoplasma-contaminated cell cultures. These sources are not of concern in the Vow manufacturing process since no animal-derived components are employed. However, the csMCB was tested for mycoplasma prior to transfer to Vow. Mycoplasma contamination can also occur via contaminated reagents, personnel, and airborne particles and aerosols (Nikfarjam and Farzaneh 2012). Therefore, tests for mycoplasma

contamination were performed by Vow on working cell banks (vWCB) cells and culture, and batches of Vow's production runs. No contamination with mycoplasma was detected in vWCB cells, nor in Vow cultured quail where the limit of detection was 10 CFU/mL.

csMCB cells were evaluated for mycoplasma contamination according to the European Pharmacopeia §2.6.7 method using quantitative PCR (qPCR). No mycoplasma was detected in either of the master bank cell samples. The following species represent an optimal selection for 2.6.7 validation according to the Pharmacopeia: *A. laidlawii*, *M. fermentans*, *M. hyorhinis*, *M. orale*, *M. pneumoniae* or *M. gallisepticum*, *M. synoviae*, *M. arginine* and *Spiroplasma citri*. Detailed reports are in Appendix 3.

Vow cultured quail were evaluated for mycoplasma contamination using the Lonza MycoAlert™ testing, which detects viable mycoplasma based on enzymatic activity, and PCR (enriched), which detects mycoplasma DNA. The Lonza MycoAlert assay detects mycoplasma specific metabolism present in most members of the mollicute family (Mycoplasma, Acholeplasma, Entomoplasma and Spiroplasma) (Lonza 2022). 95% of all mycoplasma infections of cells grown in culture are caused by just six species (*M. orale, M. arginini, M. fermentans, M. salivarum, M. hyorhinis and A. laidlawii*) (Timenetsky et al. 2006; Dvorakova et al. 2005); the MycoAlert™ Assay detects these species and many more. To date, Lonza has tested 44 species of mollicutes including species originally isolated from human, bovine, porcine, ovine, canine, murine, avian, insect and plant sources. Four individual batches of cultured quail were tested for mycoplasma; no mycoplasma was detected. Detailed reports are in Appendix 3.

B.4.1.2 Sterility testing

Sterility testing on the csMCB cells and the cell culture supernatant was performed by direct inoculation according to USP71 and European Pharmacopeia §5.2.3 guidance. In a direct inoculation test, a small volume of the product is transferred directly into culture medium before incubation to detect viable microorganisms. Specifically, thioglycollate (THIO) and Trypto-Casein-Soy (TCS) broth were used in the sterility testing. THIO and TCS are both nonselective media that enable the growth of a wide range of microorganisms, including both aerobic and anaerobic microorganisms, as well as those with complex nutritional requirements (e.g., *Streptococci, Neisseria, Brucella, Corynebacteria*, etc) [Becton, Dickinson and Company 2015, Bio-Rad 2014]. In addition to the direct inoculation sterility test, the method suitability test was performed to determine whether the product contains antimicrobial properties that would prevent the direct inoculation test from detecting the presence of viable microorganisms.

Testing for sterility of the sample in THIO and TCS media resulted in no observed microbial growth. Therefore, the csMCB cells and cell culture supernatant are concluded to be commercially sterile. A full report is included in Appendix 4.

B.4.1.3 Endogenous retrovirus

Avian species have co-existed with retroviruses for millions of years without any effect on the safety of consumption of quail or other common poultry species such as chicken (Smith et al. 1999). Integration of exogenous DNA from endogenous retroviruses into vertebrate genomes is common; the average vertebrate genome consists of 4-10% of residual viral genomes (Dunislawska et al., 2019). Studies reported to date fail to demonstrate any association between poultry retroviruses and human disease (DiGiacomo and Hopkins 1997). Therefore, similar to conventional poultry, endogenous retroviruses are not anticipated to be a food safety risk in cultured quail due to their lack of infectivity. Regardless, testing was performed to evaluate potential for retroviral contamination in cultured quail cells.

A key step of retroviral replication is reverse transcription of the RNA into the genome. This process is carried out by reverse transcriptase, an enzyme carried in retrovirus particles and encoded within the genome itself. Due to the essential role of reverse transcription in retroviral replication, retroviral contamination may be detected through the use of reverse transcriptase (RT) assays, which detect the conversion of an RNA template to cDNA due to the presence of RT enzyme when retroviruses are present in the test sample.

Detection and quantification of reverse transcriptase activity to demonstrate the absence of avian retroviruses in vMCB cells was performed according to GLP and GMP. The test sample and retrovirus particle recovery control were added with reverse transcriptase reaction mix into tubes. *Brome mosaic virus* (BMV) RNA provides a template for reverse transcriptase activity which is amplified using TaqMan® real-time PCR technology. F-PERT (Fluorescent Product Enhanced Reverse Transcriptase) analysis was performed on the test samples after amplification in an Applied Biosystems 7900HT Fast Real Time PCR system to determine the presence of reverse transcriptase activity.

Reverse transcriptase activity was below the detection limit of 2.0×10^4 retroviral particles per mL; there was no detection of viral extraneous agents or retroviruses. Therefore, the vMCB cells are concluded to not harbour retroviruses. The full retrovirus report is provided in Appendix 5.

B.4.1.4 Species-specific bacteria

Vow WCB cells were tested for a range of species-specific potentially food-borne bacteria associated with quail via PCR. This type of test is one of the most used and sensitive tests for detecting the presence of microbial genetic material. Species-specific bacteria tested include *Chlamydophila spp.,* the causative agent of avian chlamydiosis, a common systemic bacterial infection in poultry. Other bacterial contaminants including common foodborne pathogens that could be introduced through manufacturing were tested in the final product, discussed in Section B.4.1.6.

All reports were negative and no *Chlamydophila spp*. were detected. The reports of analyses are included in Appendix 6.

B.4.1.5 Species-specific viruses

Vow WCB cells were tested for species-specific viruses using PCR. vMCB cells were tested for potential food-relevant viruses including Influenza Type A (Avian influenza) and Newcastle Disease Virus via PCR. No viral genetic material was detected. All reports were negative and no adventitious agents were detected. The final report is provided in Appendix 7.

B.4.1.6 Final product testing

Table B.4.1.6-1 Microbiological testing of the final product

Microbiological	**Specification**	**Results**					
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Standard plate count (CFU/g)	< 10^4	<100	<100	<100	<100	<100	<100
Coliforms (CFU/g)	< 100	<10	<10	<10	<10	<10	<10
E. coli (MPN/g)	<3	<3	<3	<3	<3	<3	<3
Enterobacteriaceae (CFU/g)	< 100	<10	<10	<10	<10	<10	<10
Salmonella (/25g)	Not detected in 25 g	ND	ND	ND	ND	ND	ND
Listeria monocytogenes (/25g)	N/A	ND	ND	ND	ND	ND	ND
Coagulase positive *Staphylococcus aureus* (CFU/g)	N/A	<10	<10	<10	<10	<10	<10

ND = Not detected

No *E. coli*, *Salmonella*, *Listeria monocytogenes or* coagulase positive *Staphylococci* were detected in Vow cultured quail. Standard plate count, coliforms, and *Enterobacteriaceae* are within specifications. The results from microbiological analysis of six batches of product are presented in Table B.4.1.6-1.

There are no microbiological limits established for similar products, such as poultry, in the Australia New Zealand Food Standards Code (Schedule 27). The microbiological criteria were derived from microbiological standards for ready-to-eat food (articles of food made available for

sale for direct human consumption without the need for cooking or any other form of processing to eliminate, or reduce microbes) as listed in the Compendium of Microbiological Criteria for Food (FSANZ 2022). The most conservative values were 1.0 x 10^5 CFU/g for standard plate count, 1.0 x 10^4 CFU/g for *Enterobacteriaceae*, 1.0 x 10^2 CFU/g coagulase positive *Staphylococcus aureus*, < 3 MPN/g for *E. coli*, and 'not detected' for *Salmonella* and *Listeria monocytogenes*. Coliforms were measured as indicator organisms to evaluate the microbiological status of food.

The microbiological data reports are provided in Appendix 8.

B.4.2 CHEMICAL ANALYSIS

B.4.2.1 Heavy metals

Three batches of Vow cultured quail were tested using ICP-MS for heavy metals (antimony, arsenic, cadmium, lead, mercury, and tin) (Table B.4.2.1-1).

Table B.4.2.1-1 – Results of heavy metal testing (mg/kg)

Heavy metals	**Results**		
	Batch 1	Batch 2	Batch 3
Antimony	<0.01	<0.01	<0.01
Arsenic	<0.05	<0.05	<0.05
Cadmium	<0.01	<0.01	<0.01
Lead	<0.01	<0.01	<0.01
Mercury	<0.01	<0.01	<0.01
Tin	<0.02	<0.02	<0.02

No heavy metals were detected in Vow cultured quail (Table B.4.2.1-1). The heavy metal testing reports are provided in Appendix 9.

B.4.2.2 Antibiotics

Antibiotics are employed only during the initial cell culture development stage of the quail embryo cells. Antibiotics were used only for the first two passages of the primary culture established from the quail embryo. Starting with passage 3, the medium did not contain any antibiotics [detailed information is provided in the Certificate of Analysis in Appendix 10]. The cells were cultured for more than 12 months in the absence of antibiotics before entering vMCB production. No antibiotics were used after the csWCB was received by Vow.

To confirm that there are no antibiotic residues in the final product, testing for antibiotic residues in the cultured quail was performed. No antibiotic residues were detected in Vow cultured quail.

The maximum residue limits for the antibiotics used in Australia are listed as 60 ppb penicillin in mammalian meat and 300 ppb of Streptomycin in mammalian meat (Australia New Zealand Food Standards Code, Schedule 20 – Maximum residue limits). Vow cultured quail was tested and demonstrated to have no detectable penicillin nor streptomycin antibiotic residue above the detection limit of 0.01 mg/kg and 0.1 mg/kg, respectively. The residue report is provided in Appendix 10.

B.5 Manufacturing process for a novel food ingredient

B.5.1 Manufacturing process

Vow cultured quail is prepared in stages:

1. Preparation of Master Cell Bank at cell-line supplier facility (csMCB)
2. Preparation of Working Cell Bank at cell-line supplier facility (csWCB)
3. Preparation of Vow Master Cell Bank (vMCB)
4. Preparation of Vow Working Cell Bank (vWCB)
5. Cell Expansion (seed train)
6. Harvest

All materials used in the production of Vow cultured quail have been evaluated and determined to meet the requirements for food grade or pharmaceutical grade ingredients of a purity and quality suitable for their intended use in food. Processing conditions are designed for food production following HACCP principles. All equipment undergoes routine cleaning, maintenance and care.

A Working Cell Bank was sourced from a cell-line supplier (termed ‘csWCB’). The cell-line supplier is a European biotechnology company that provides fully characterised cell banks that are GMP compliant and tested according to international guidelines for use in vaccine development (US FDA cGMP – 21 CFR, European Pharmacopoeia and ICH guidelines). Once the csWCB is received, Vow cultured quail manufacturing is performed at the Vow according to Standard Operating Procedures (SOPs) that are periodically reviewed and updated as needed. Additionally, manufacturing occurs in accordance with Vow’s Food Safety Plan, including HACCP.

A schematic overview of the cell development work at Vow’s cell-line supplier and the manufacture of cultured quail at Vow is provided in Figure B.5.1-1. Appendix 11 provides more details on the manufacturing process at the Vow facility, after receipt of the csWCB.

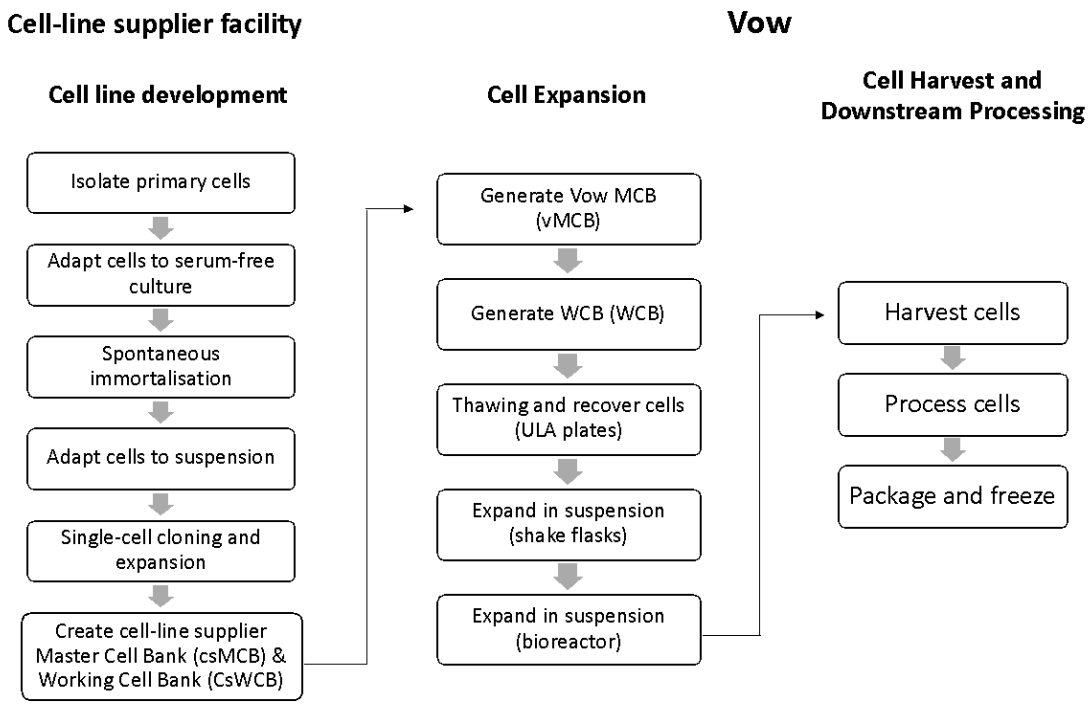

Figure B.5.1-1. Schematic overview of the cell development, cell expansion, and cell harvest and downstream processing of Vow cultured quail.

Preparation of Master Cell Bank and Working Cell Bank at Cell-line Supplier

The cell-line supplier Working Cell Banks (csWCBs) used by Vow were obtained from a European biotechnology company. An official Veterinary Certificate verifies that the farm where quail were sourced is 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 animal owner that could indicate an infection. Additionally, a microbiological examination of the eggs from the egg packing station in 2018 did not reveal any evidence of salmonella. Therefore, it is concluded that the source animal was healthy and free from disease. The Official Veterinary Certificate is provided in Appendix 12.

The cell line was developed and produced in a research laboratory under controlled conditions, following defined Standard Operating Procedures. All work steps, operators, dates, equipment, and reagents used, certificates of analysis, *etc.* were documented. Access restrictions and dedicated rooms for quail experiments were in place.

Primary cells were obtained from the quail embryo by digestion and adapted to growth in suspension culture by serum dilution. Spontaneous mutations in the quail cell population are the mechanism of immortalisation. No viral sequences and no foreign genes were introduced. Therefore, the cell line is not regarded as being genetically modified (GM). Cells were then adapted to growth in suspension in serum-free suspension cell growth medium (SCGM) by cultivating them in a spinner flask for an extended period.

Preparation of Vow Master Cell Bank and Working Cell Bank

Cryopreserved csWCB quail fibroblast suspension cells are thawed to generate the vMCB and vWCB, respectively. The freezing medium is removed by centrifugation, supernatant is aspirated, and the remaining cell pellet is resuspended in culture medium and transferred to a well plate. Cell counts and viability are determined, followed by recovery for 24 hours prior to subculturing.

Recovered csWCB are subcultured into fresh culture media in ultra low attachment plates. Cell counts and viability are monitored daily until cells reach the target density (cells/ml). The cells undergo a second round of subculturing at a 500 mL final volume before cryopreservation as vMCB vials.

Once cells reach the target density, cells are centrifuged and resuspended in freezing medium. vMCB and vWCB vials are frozen at -80°C for 16-24 hours, then stored at < -135°C in liquid or vapour phase nitrogen.

Cell Expansion

Cells are expanded through multiple phases, with increasing volume. The cultures are monitored daily, taking cell count, pH measurement, and dissolved oxygen measurement. Routine inspection for microbial contamination is performed, including visual (looking for turbidity or films) and olfactory inspection, and inspection under light microscope (observation for bacteria or fungal contamination).

Thawing and preparing cells, cell expansion

Cryovials of vWCB cells are thawed and transferred into a shake flask or small bioreactor. Cells are incubated in each vessel until harvest. The shake flask culture is monitored daily until the cells reach a target cell density.

Passaging cells for cell expansion

Cells are passaged into a 10x greater culture volume at each passage. The culture is monitored daily until the cells reach a target cell density in each flask.

Seeding Stirred Tank Reactor (STR) for cell expansion

Culture from several flasks are combined and transferred into a media bottle subassembly.

Growth medium is filtered into a Stirred Tank Reactor (STR). The STR is then aseptically connected to bioprocessing containers filled with additional growth medium components (*e.g.*, components used for pH stabilisation, growth promotion and antifoaming). Cells begin

proliferation in a STR, and after reaching targets for cell culture volume and density, the following may then occur:

1. Complete harvest: all cells in the STR are harvested;
2. Draw and fill: a portion is harvested and a portion is left as cell inoculum to top up the bioreactor again to a target volume.

As required by production demand, a larger bioreactor (*e.g*., 2000L STR, 20,000L STR) may be seeded after either option (1) or (2) outlined above.

The process is repeated for larger vessels.

Harvest

Suspension cells are harvested by concentration and centrifugation.

The remaining dewatered cells may be mixed with food-grade additives, and is then transferred to a clean container for freezing and storage. The centrifugation, processing, packaging, freezing and storage are completed in a food-preparation facility with temperature and humidity control as well as HEPA-filtered overpressure. The post-harvest steps reflect common techniques used in food processing and can be found in our HACCP Food Safety Plan, in Appendix 13.

Cleaning

After harvest, the process equipment is washed with a cleaning agent through a clean-in-place loop, by continuously running the cleaning agent through the lines, vessels, centrifuge and any other equipment, then to waste disposal. The process is then repeated with tap water. Any single-use materials are disposed of after use.

B.5.2 Quality Control

Vow cultured quail is currently manufactured in a factory designed for low contamination risk, following Standard Operating Procedures. Vow implements a Hazard Analysis and Critical Control Point (HACCP) based food safety management system to control potential food safety hazards. The full HACCP plan, including the hazard analysis, flow chart, risk matrix, CCP decision tree, HACCP audit table, and critical limit validation can be found in Appendix 13. Commercial production will also be in compliance with Good Cell Culture Practice (GCCP) The GCCP plan is included in Appendix 14.

B.6 Specification for identity and purity for a novel food ingredient

A published specification is not available for cultured quail. Vow proposes specifications that establish the qualitative and quantitative parameters for each batch of cultured quail.

B.6.1 Proposed Product Specifications

The proposed production specifications for Vow cultured quail are presented in Table B.6.1-1.

Table B.6.1-1 Proposed specification

Parameter	**Specification**
Proximate	
pH	> 4.5
Protein (%)	> 4
Moisture (%)	> 80
Ash (%)	< 1.5
Fat (%)	0.5 - 3
Carbohydrates (%)	< 1
Microbiological	
Standard plate count (CFU/g)	$< 10^4$
Coliforms (CFU/g)	< 100
Escherichia coli	< 3 MPN/g
Enterobacteriaceae (CFU/g)	< 100
Salmonella	Not detected in 25 g

B.6.2 Batch analyses

The analysis of three batches for proximate testing and six batches for microbiological testing demonstrates consistency of the manufacturing process and compliance with the standards. Proximate testing results are presented in Table B.6.2-1. Microbiological batch testing results details are provided in section B.4.1.6.

Table B.6.2-1 Batch analyses – proximate testing

Proximate	Results		
	Batch 1	Batch 2	Batch 3
Protein (g/100g)	9.5	8.6	10.5
Moisture (g/100g)	86.7	88	85.6
Ash (g/100g)	1	0.9	1.1
Fat (g/100g)	1.6	1.4	1.5
Carbohydrates (g/100g)	1	1	1

B.7 Analytical method for detection of a novel food ingredient

Cultured quail may be detected through species identification (as described in Section C.6.1.2).

C. Information on the safety of the novel food for 'food ingredients derived from a new source' and 'foods produced by a process not previously applied to food'

Information on the safety of Vow cultured quail is described in this section. This section is completed in accordance with the information requirements in relevant sections of Guideline 3.5.2. (Novel Foods) Sections C.6 (Food ingredients derived from a new source) and C.7 (Foods produced by a process not previously applied to food) of the *Food Standards Australia New Zealand Application Handbook (2019)*.

A multi-tiered testing strategy was developed to establish the safety of Vow cultured quail incorporating current guidance from FSANZ. This section establishes the safety of Vow cultured quail by:

1. Confirming identity (Section C.6.1.2);
2. Describing a long and safe history of consumption of conventional quail (Section C.6.1.3);
3. Demonstrating the absence of allergenicity (Section C.6.1.4);
4. Performing an analysis of the gross composition and nutrient content (Section C.6.2.1);
5. Performing a comprehensive risk analysis of the inputs and processing aids (Section C.6.3.1 – C.6.3.3); and
6. Performing a whole genome assessment of the genetic stability of the cell lines (Section C.6.3.4).

C.6.1 Information on the safety of the source organism

C.6.1.1 Identity

Vow cultured quail is derived from Japanese quail (*Coturnix japonica*), a species domesticated and consumed by humans for thousands of years. The taxonomic identity of cultured quail is presented in Table C.6.1.1-1.

Table C.6.1.1-1. Taxonomic identity of cultured quail

Kingdom	Animalia
Phylum	Chordata
Class	Aves
Order	Galliformes
Family	Phasianidae
Genus	*Coturnix*
Species	*Coturnix japonica*

C.6.1.2 Species confirmation

Species identification was performed on the csMCB and vMCB cells to confirm species of the cells. The analysis for csMCB cells was performed by the cell-line supplier. The analysis for vMCB cells was performed by an external test facility commissioned by Vow. csMCB and vMCB cells were confirmed to be *Coturnix japonica* (Japanese quail).

Briefly, species identification was performed through DNA barcoding using the mitochondrial cytochrome C oxidase subunit 1 (COI) sequence as a marker gene (Hebert *et al.*, 2003, Tizard *et al.* 2019, Folmer *et al.*, 1994). COI sequencing is broadly accepted as an effective way to identify the species of an unknown sample and has been validated by the ATCC for use with cell lines (Cooper *et al.*, 2007), by the FDA to identify species of seafood for proper labelling (FDA 2011), and by others for forensic applications (Dawnay *et al.*, 2007). Primers were designed to match the COI sequence. Using the cell total DNA as a template, PCR with these primers resulted in a single PCR product. Sequencing of the PCR product yields a high-quality sequence covering the complete mitochondrial cytochrome C oxidase subunit I gene. Sequences are analysed using the Barcode of Life Data System informatics workbench (BOLD; Ratnasingham and Hebert, 2007) and/or BLAST (NCBI).

The analyses demonstrate 100% identity of the csMCB and the vMCB cells with *C. japonica* as described in Appendix 15.

C.6.1.3 General history of safe quail consumption

Quail has been an important source of meat throughout history and continues to be raised commercially and hunted for meat globally. Native peoples in Africa and the Palearctic Ecoregion counted on the seasonal abundance of huge migrating flocks. In recent years hunting

has been impacted in some areas as wild population numbers plummet due to habitat loss. However, farming of domesticated quail, *Coturnix japonica*, or Japanese quail, has dramatically increased, with the main production of meat occurring in China, Europe, and the United States, along with well-developed industries in other countries, such as Australia, Canada, Egypt, Saudi Arabia, India, Estonia, Russia, Singapore, Venezuela, Peru, Columbia, and Bolivia (Lukanov 2019, Katerynych & Pankova 2020). The available data indicate world *C. japonica* meat production is estimated to be 200,000 to 240,000 tonnes, or about 0.2% of the world poultry meat produced (Lukanov 2019).

A comprehensive literature search was conducted and did not identify any published literature to suggest that consumption of cultured quail or cultured poultry is associated with allergic, toxic, or adverse health effects. Appendix 16 contains additional details on the literature search. There are few reports of human illness due to consumption of hunted quail (Korkmaz *et al.* 2011). Coturnism is the most widely reported illness, where humans can be poisoned from consuming European migratory quail. This type of poisoning is not possible from cultured *C. japonica* quail. The disease is extremely rare and has only been reported after consumption of wild quail from the *Coturnix genus* eaten during the migratory season. It is specifically related to the diet of the quail, where they eat toxic plants and seeds that are then metabolised into the body of the quail (Korkmaz *et al.* 2011; Lewis *et al.* 1987). Similar to other intensively reared galliform birds, conventional quail meat is susceptible to contamination by *Salmonella, E. coli,* and *Campylobacter* from the gut or faeces during slaughter. Microbes from these sources are not anticipated to be present in cultured quail as samples are biopsied under controlled conditions, and cells are tested for sterility prior to use in manufacturing. Regardless, testing for these microbes in the harvested cells is performed to confirm that there is no residual nor introduced contamination during the manufacturing process.

Australia and New Zealand

Australia's quail farming industry started in the early 1970s, with one of the largest producers of quail in the country (Game Farm) being established in 1975 (Scolexia Animal and Avian Health Consultancy, 2009). The most common species of quail used in commercial enterprises is the Japanese quail (*Coturnix japonica*).
In 2003 an annual harvest of 6.5 million farmed quail in Australia was reported, with the majority of production located in New South Wales (Scolexia Animal and Avian Health Consultancy, 2009, AgriFutures Australia: Game Birds, 2017). The Australian Bureau of Statistics reported a national poultry flock on June 30, 2021 that included 111 million chickens raised for meat (up 10% from 2020), and 3 million other poultry (*e.g.,* geese, turkeys, quail and ducks) (Australian Bureau of Statistics 2022).

Quail is the smallest species of game bird farmed in Australia, but the largest by volume (AgriFutures Australia 2017). In 2009, there were approximately 10 producers of quail across Australia (Scolexia Animal and Avian Health Consultancy, 2009). The Game Management Authority in Victoria estimates hunters kill and harvest about 175,000 quail a year. New Zealand has several commercial quail farms, and quail are also hunted in the wild. Early European settlers introduced 29 species of upland game birds to New Zealand, including quail, which

continue to thrive in huntable populations (Fish and Game New Zealand, n.d.). In addition to satisfying domestic consumption, Australian companies export quail meat to Asia (Da Cunha, 2009) and one exporter is authorised to import quail into Singapore (Game Farm Pty Ltd).

Asia

Quail is a part of traditional cooking throughout Asia and is frequently found in restaurants. In Singapore alone, approximately 80,000 quail are slaughtered for meat each year (SFA 2021). Quail meat is readily available in several stores and more than 20 restaurants (Yip, 2015). Quail are both imported and raised on farms in Singapore. At least two quail farms operate in Singapore, including the Lian Wah Hang Farm Pte Ltd Quail and Poultry Farm, established in 1954. Lian Wah Hang Farm has >45,000 quail producing 25,000 eggs per day and 2,500 pieces of meat per week, which are sold direct to consumers and in the local markets (personal communication 18 May 2022, William Ho, owner of Lian Wah Hang Farm).

Outside of Singapore, China is the world's largest quail meat producer, with an estimated annual production of 146-190 thousand tonnes of *C. japonica* meat (Da Cunha, 2009). In Malaysia, the 2009 demand for quail had increased 20-25% each year since 1995 (The Edge 2009). In Saudi Arabia, a single farm produced 1.5 thousand tonnes of quail in 2019 (Riyadh & U.S. Embassy, 2020).

North America

In the United States (USA), there are sales of over 22 million quail from 3,061 farms, predominantly in the states of Georgia, Alabama, and Texas (USDA Census of Agriculture, 2017). Commercial quail meat production is also popular in Canada. Canadian production was estimated at 829,784 birds in 1991 (Kermode 1997), with British Columbia alone producing 2 million quails per year by 2000 (Da Cunha 2009).

Europe

It is estimated that 4.2 million quail were hunted for meat in Europe in 2009 (Perennou, 2009). Wild populations have plummeted since that time, but quail farming has increased along with the popularity of this meat. Unofficial reports estimate that 100 million quail are reared in the EU, mostly for meat production (Lukanov 2019). Most of the farmed quail finds markets within Europe, although there is some export to Asia, including export of frozen *C. japonica* from France to Singapore. A 2009 report in Poultry World provides snapshots of the largest producers in Europe (Da Cunha, 2009) (Table C.6.1.3-1).

Table C.6.1.3-1. Largest quail producers in Europe

Country	Quail meat production (tonnes)	Year
Spain	9,300	2004
France	8,197	2006
Italy	between 3,300 and 3,600	2009

Portugal	960 and 1,600	2009

Africa

Between 2008 and 2012, an average of 2 million wild quail were captured annually along the Mediterranean coast of Egypt, with numbers jumping to 3.3. million in 2012 (Eason *et al.*, 2016). Quail may have once been primarily caught for subsistence— and many Bedouin are still subsistence hunters— but wild-caught quail is also sold in markets as a seasonal luxury food. By 2021, six large-scale farms, along with hundreds of small-scale farmers, were producing various quail breeds in South Africa, Kenya, Uganda, and other African countries (Mnisi *et al.*, 2021).

C.6.1.4 Assessment of allergenicity

The allergenicity assessment follows a three part strategy of:

1. Literature review;
2. Protein sequence analysis; and,
3. Allergen residue analysis.

Literature review

A review of the public scientific literature uncovered no reports that implicate Japanese quail, or other species of quail, with allergenicity in humans. Details on the literature review are provided in Appendix 16. The long history of consumption of quail in combination with a lack of reports of allergenicity offers strong evidence for the safety of quail protein as a food product. Regardless, a comparative protein analysis of the proteins identified in *C. japonica* was performed using the publicly available annotated genome (GenBank/RefSeq GCA_001577835.2) to confirm these results.

Sequence homology

Assessments were undertaken to determine whether the amino acid sequences of protein from 1) *C. japonica*, and 2) Vow cultured quail had similarity to any known allergens. The annotated reference genome of *C. japonica* was evaluated to confirm that no known food allergens are encoded in the *C. japonica* genome; this analysis was used as a basis to assess for potential allergens in Vow cultured quail.

Protein sequences were compared to the sequences on the AllergenOnline database (version 21, released on February 14, 2021). An initial screen of all Japanese quail proteins was performed against the AllergenOnline database v21, searching for full-length alignments by FASTA (a text-based format that represents amino acid sequences), with identity matches greater than 50% indicating possible cross-reactivity. This screen identified 80 protein sequences with potential matches. Each of these proteins were then evaluated using a sliding window of 80 amino acid segments of each protein to find sequence identities greater than 35%

(according to FAO/WHO CODEX Alimentarius guidelines, 2004). It has been suggested that this threshold be considered in conjunction with *E*-scores (expectation scores) generated from the FASTA algorithm to make a more informed decision regarding whether a protein has the potential for allergenic cross-reactivity (Abdelmoteleb *et al.* 2021, Thomas *et al.*, 2005; Ladics *et al.*, 2008; Silvanovich *et al.*, 2009; Cressman *et al.*, 2009). The *E*-score reflects the measure of relatedness among protein sequences and can help separate the potential random occurrence of aligned sequences from those alignments that may share structurally relevant similarities. A small *E*-score (*e.g.*, less than $1e^{-7}$) reflects a possible functional similarity and may suggest a biologically relevant similarity for allergy or potential cross-reactivity, while large *E*-scores (>1.0) are typically associated with alignments that do not represent a biologically relevant similarity (Pearson 1999, 2016; J. G. Henikoff & Henikoff, 1996; S. Henikoff & Henikoff, 1992, Russell, 2014). Therefore, sequences with an E-score of $<1e^{-7}$ were further evaluated to assess their potential allergenicity in Appendix 17.

Appendix 17 lists the protein-coding sequences in *C. japonica* found to have similarity with related proteins from a variety of organisms. One of the challenges of evaluating an entire genome is that identity matches greater than the CODEX limits are common for many proteins that are conserved through extensive evolution but are not predictive of published allergy risks based on observed taxonomic cross-reactivity (Abdelmoteleb *et al.* 2021). Most 'allergenic' proteins with sequences similar to those of *C. japonica* proteins of interest are ubiquitous, essential for proper physiological function, are highly conserved across diverse species, and are not known to be toxic. The sequence-related putative allergens identified in this search were not common allergens and importantly none were any known to be allergenic when ingested from quail. These are discussed in this section.

Egg allergens

Several sequences related to egg allergens, including major egg proteins lysozyme C, ovomucoid, vitellogenin-1, and serotransferrin (ovotransferrin), were identified as potential allergens. Since these proteins are only expressed in eggs and not meat, they are not considered major allergens in quail meat. Further, a quantitative analysis of the egg allergen ovomucoid (one of the main allergens in egg white), using a sandwich enzyme-linked immunosorbent assay (ELISA) determined that there was no egg protein detected in Vow cultured quail.

Parvalbumins, Enolases, and Aldolases

Parvalbumins are involved in relaxation of fast twitch muscle fibers (Celio and Heizmann, 1982) whereas enolases and aldolases are glycolytic enzymes involved in energy metabolism (Diaz-Ramos *et al.*, 2012, Berridge et al, 2013), all of which are found in many species. Allergenicity in fish is found with β-parvalbumins, while in frog and chicken (and likely quail), it is associated with α-parvalbumins (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014). α-Parvalbumins are one of the primary allergens in poultry meat (Wanniang *et al.* 2022). Some antibodies react with both α- and β-parvalbumins, and rare cases of cross-allergy between fish and chicken (Gonzalez-de-Olano *et al.*, 2012) and between fish and frog (Hamada

et al., 2004; Hilger *et al.*, 2004) have been reported, claimed to be caused by cross-reactivity between fish β-parvalbumin and chicken and frog α-parvalbumins, respectively. In addition to parvalbumins, enolase, and aldolase have recently been identified as potential cross-reactive allergens (Kuehn *et al.* 2016). An analysis of *C. japonica* demonstrates that quail parvalbumins and enolases share high sequence similarity to fish (70.6% - 88.6%) and chicken (87.3% - 98.2%) proteins (Appendix 17).

The interpretation is that an individual allergic to chicken has a high probability of being allergic to quail. Individuals allergic to fish may be allergic to quail, as fish and chicken meat have been identified as cross-reactive foods. Both fish-allergic and chicken meat-allergic patients may be at risk of developing a food allergy to chicken meat or fish, respectively (Kuehn *et al.* 2016). Only a single case of clinical cross-reactivity between fish and chicken meat has been described in literature (Kuehn *et al.* 2016) and is considered rare (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014), while no reports of cross-reactivity between fish and quail have been described; therefore, there is a low probability of this occurrence.

Myosin

Myosin light chain 1 (Myl1) is necessary for adequate skeletal muscle function and is involved in many functions within muscle including contraction, cytokinesis, cargo transport, and cell migration (Heissler and Sellers, 2014, Lowey 1993). The primary structures for binding calcium are conserved in many eukaryotic species (Foth, 2006). Myl1 is one of the primary allergens in poultry meat allergies and shows a strong degree of homology with other poultry, including turkey, duck, and goose; therefore, individuals primarily sensitised to chicken meat may be anticipated to develop cross-reactive allergic reactions to quail.

Collagen

Collagen is the major structural protein in skeletal muscle with type I and type III being the most abundant. Collagen alpha 2(1) is a component of type I collagen and has evolutionarily conserved sequences across species (Gillies and Lieber, 2011, Yamada et al, 1984, Chu et al, 1984). Quail collagen alpha 2(I) chain had a 63.80% match to Barramundi (*Lates calcarifer*); although fish collagen has been proposed to be an allergen based on IgE-binding studies and two clinical case reports (Sakaguchi *et al.*, 1999; Sakaguchi *et al.*, 2000; Hamada *et al.*, 2001; Kuehn *et al.*, 2009), data from two double-blind, placebo-controlled food challenge studies in fish-allergic patients (Andre *et al.*, 2003; Hansen *et al.*, 2004) suggest that its clinical importance is very limited (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014).

Other allergens – fish allergens

Fish allergens belong to several different protein families; cross-reactivity between different species of fish is dependent on evolutionary conservation and the isoform of the protein (Kalic

et al., 2021). Major fish allergens were discussed in the previous section, however, many minor fish allergens have also been identified:

1. Creatine kinase (B-type isoform X1)
2. Triosephosphate isomerase
3. Pyruvate kinase (PKM isoform X2)
4. Lactate dehydrogenase (A chain)
5. Glucose 6-phosphate isomerase,
6. Glyceraldehyde-3-phosphate dehydrogenase

These proteins are all involved in energy metabolism and were identified by Ruethers *et al.* (2021). Due to their essential role in metabolism, these proteins are found in almost every organism that utilises glycolysis.

Creatine kinase is found in the muscle tissue of vertebrates and is necessary for cellular metabolism (Kalic *et al.*, 2021). It has been identified as a major allergen in catfish and in salmon (Ruethers *et al.*, 2021). Creatine kinase has also been implicated in pork allergy (Barbarroja-Escudero *et al.*, 2019). However, there is no literature on cross-reactivity between this allergen and poultry sources. In fact, those patients who were allergic to pork were able to tolerate other meats including poultry and fish so the potential for cross-reactivity is apparently minimal.

Triosephosphate isomerase is also important in glycolysis and is found in many species including bacteria, fungi, plants, and animals (Kalic *et al.* 2021). It is a minor catfish allergen and other saltwater products including crayfish (Ruethers *et al.* 2021, Yang *et al.*, 2017). It was also reported as a food allergen in some plant-derived foods including wheat and watermelon. Literature has shown that there is IgE cross reactivity with filamin-C, an actin binding protein (Yang *et al.*, 2017). This cross-reactivity is of interest, because filamin-B was also identified during analysis of sequence homology to quail. Filamin B and C have similar structures (Nakamura *et al.*, 2011). However, the cross-reactivity between triosephosphate isomerase and filamin C was found between two crayfish proteins. There is no evidence that this cross-reactivity would occur between quail, as fish and meat cross-reactivity is extremely rare. Additionally, while triosephosphate isomerase has been reported as a chicken allergen in dogs, there is no clinical evidence of chicken triosephosphate isomerase allergy in humans (Wanniang *et al.*, 2022).

Pyruvate kinase, glyceraldehyde-3 phosphate dehydrogenase and lactate dehydrogenase are all conserved enzymes involved in catalysing glycolysis and are all minor fish allergens (Kalic *et al.*, 2021). There is evidence of cross-reactivity of pyruvate kinase, glyceraldehyde-3 phosphate dehydrogenase and lactate dehydrogenase between fish and chicken in dogs with allergic skin disease (Bexley *et al.*, 2019). Lactate dehydrogenase A has also been implicated in red meat allergy (Wilson and Platts-Mills, 2018). However, red meat allergy is very rare and there is no relation between this allergy to red meat and allergy to poultry (Hemmer *et al.*, 2016).

Glucose-6 phosphate isomerase is another enzyme related to energy metabolism and identified as a minor seafood allergen (Kalic *et al.*, 2021). The study by Ruethers *et al.* (2021) was the first to report this fish allergen.

All the proteins in this section are minor seafood allergens. Minor allergens are defined by IgE binding frequency at less than 50% IgE binding, meaning response to these allergens is less common. While those with fish allergy risk developing an allergy to chicken and vice versa, this is also a rare occurrence (Kuehn *et al.* 2016, EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014). There is also no strong relation between red meat allergy to these proteins and poultry meat allergy (Hemmer *et al.*, 2016). Additionally, no cases of cross-reactivity between fish allergy and quail have been reported. Thus, it is unlikely that individuals will be allergic to quail provided they are not allergic to these proteins in chicken.

Summary

The sequence analysis found there were no sequences likely to be expressed in quail meat that are associated with the common allergens in either *C. japonica* or Vow cultured quail, including cereals containing gluten (wheat, rye, barley, oats), crustacea and crustacean products, eggs and egg products, fish and fish products, lupin, peanuts, soybeans, and their products, milk and milk products (including lactose), tree nuts and nut products. However, there may be some cross-reactivity for fish-allergic or chicken-allergic patients. Due to the long history of consumption of quail without any reports of allergenicity to quail meat, it is not expected that there are any novel allergens. Similar to chicken or other poultry, some people may experience allergic reactions, but it is relatively rare and therefore poultry allergens are not considered a major allergen. Regardless, follow-on testing for these common allergens was performed to demonstrate that no residues of major allergens were detected.

Allergen residue testing

Enzyme linked immunosorbent assay (ELISA) analyses were performed on Vow cultured quail to determine the presence of any major allergens including: gluten, soy protein, egg, peanut, walnut, hazelnut, almond, beta-lactoglobulin (milk), casein (milk), total milk allergens, crustacean (tropomyosin), mollusc, cashew kernel, macadamia nut, pistachio, Brazil nut, sesame seed protein, mustard seed protein, fish, lupin, pine nut, sulphites, and pecan nut. None of these residues were detected. Therefore, while the detection of some genetic sequences indicated potential expression of fish and seafood allergens, the allergen residue testing confirms that these proteins are not present in the final product. Appendix 18 includes the ELISA full allergen residue results.

C.6.2 Information on the composition of the novel food ingredient derived from a new source

C.6.2.1 Gross composition and nutrient analysis

The composition of raw samples of conventional quail (*C. japonica*, as well as a closely related species, *Colinus virginianus*, Northern Bobwhite quail), Vow working cell back (vWCB) samples, and Vow cultured quail was measured and compared to assess the similarities in gross composition. Conventional quail was sampled from commercially available quail that are raised for consumption. Tissues were sampled from three different *C. japonica* sourced in Australia, three different *C. japonica* sourced in Singapore, and three different *C. virginianus* quail (Table C.6.2.1-1). Testing was performed in accredited labs. Appendix 19 provides detailed information on each lab, while Appendix 20 includes information on methods, including limit of reporting, limit of detection, and measurement uncertainty. Appendix 21 includes all proximate test reports.

Table C.6.2.1-1 Conventional quail – Sourcing information

Sample	Date sampled	Source	Tissue
C. japonica (sourced in Australia)	07/07/2022	Australia	*C. japonica quail meat (Samples from 3 different quail)*
C. japonica (sourced in Singapore)	23/07/2022	Singapore	*C. japonica quail meat (Samples from 3 different quail)*
C. virginianus (sourced in Australia)	15/06/2022	Australia	*C. virginianus quail meat*
	11/06/2022	Australia	*C. virginianus quail meat*
	09/06/2022	Australia	*C. virginianus quail meat*

Gross composition and nutrient analysis included proximate testing, amino acids, minerals, vitamins, and fatty acids analysed in all samples. Values from literature ('reported values') are also included for comparison where appropriate. Peer-reviewed literature and government reference databases were mined for information on quail meat, including the Food Standards Australia New Zealand (FSANZ) Australian Food Composition Database, the Singapore Health Promotion Board (HPB) Energy and Nutrient Composition of Food database, the United States Department of Agriculture (USDA) FoodData Central Database, Czech Food Composition Database, French Food Composition Table (Ciqual), Indian Food Composition Tables, Japan Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Standards Food Composition, Slovak Food Composition Database, and the Turkey National Food Composition Database included as "Reported values" in the proximate, amino acid, mineral, vitamin, and fatty acid composition data tables (Tables C.6.2.2-1, C.6.2.3-1, C.6.2.3-2, C.6.2.4-1, C.6.2.5-1, and C.6.2.6-1). The full literature and government database review is provided in Appendix 22.

C.6.2.2 Proximate profile

Vow cultured quail is principally composed of moisture (86.8 ± 1.2%) and protein (9.5 ± 1.0%), with some fat (1.5 ± 0.1%) and carbohydrates (1.0 ± 0.0%). Vow cultured quail is composed primarily of fibroblasts, whereas conventional quail was sampled from full tissue (*i.e.,* muscle, fat, cartilage, connective tissues); therefore, the percentage of fat in cells is lower than in tissue of conventional quail meat. Ash content (minerals and trace elements) of Vow cultured quail is similar to that of conventional quail (1.0 ± 0.1%).

Table C.6.2.2-1 Proximate results

	Ash (g/100g)	**Protein (g/100g)**	**Fat (g/100g)**	**Carbohydrates (g/100g)**	**Moisture (g/100g)**	**Gross energy (kJ/100g)**
Vow cultured quail	1.0 ± 0.1	9.5 ± 1	1.5 ± 0.1	1 ± 0	86.8 ± 1.2	233 ± 20.8
Conventional quail						
Au *Coturnix*	1.1 ± 0.2	18.2 ± 1	8.9 ± 3.2	<1	71.8 ± 2.7	640 ± 102
Au *Colinus*	1.1 ± 0.1	21.2 ± 0.1	2.9 ± 2	<1	76.2 ± 1.6	465 ± 77.8
Sg *Coturnix*	1.2 ± 0.2	18.8 ± 0.6	9.1 ± 1.1	2.1 ± 0.5	68.9 ± 0.9	693 ± 37.8
Reported conventional values						
Minimum-Maximum	0.9 – 2	15.6 – 27	1.3 – 12.9	0 – 0.1	50.9 – 78.2	515 - 870

C.6.2.3 Amino acids

Amino acid values are normalised to protein content, as measured in the proximate analyses. The quantities and distributions of most amino acids in Vow cultured quail fall within the range measured in conventional quail (Table C.6.2.3-1). Where amino acids in Vow cultured quail exceed 10% of the range of conventional quail, a detailed evaluation was performed to assess safety implications. The raw amino acid measurements are provided in Appendix 21, and the statistical analysis is provided in Appendix 23.

Table C.6.2.3-1 Amino acid results (g/100g protein)

	Cultured quail	**Conventional Quail**	
		Au *Coturnix*	Sg *Coturnix*
Histidine	4.2 ± 0.12	3.2 ± 0.010	2.64 ± 0.0349
Isoleucine	4.9 ± 0.038	5.3 ± 0.43	4.51 ± 0.0676
Leucine	9.0 ± 0.065	8.8 ± 1.7	7.55 ± 0.135
Lysine	8.5 ± 0.45	12 ± 1.1	8.25 ± 0.371
Methionine	2.3 ± 0.31	3.2 ± 0.22	2.67 ± 0.0294
Phenylalanine	4.8 ± 0.048	4.5 ± 0.41	3.75 ± 0.0844
Threonine	4.8 ± 0.016	5.2 ± 0.43	4.19 ± 0.0686
Tryptophan	1.6 ± 0.035	0.93 ± 0.11	Not measured
Valine	5.7 ± 0.049	5.6 ± 0.66	4.59 ± 0.0841
Serine	5.8 ± 0.061	4.5 ± 0.35	3.80 ± 0.0747
Glycine	5.6 ± 0.12	5.3 ± 0.57	4.26 ± 0.0795
Alanine	5.6 ± 0.033	6.8 ± 0.68	5.24 ± 0.172
Tyrosine	4.1 ± 0.062	3.8 ± 0.37	3.29 ± 0.0750
Arginine	7.1 ± 0.045	7.0 ± 0.53	7.14 ± 0.0500
Glutamic acid, glutamine	15 ± 0.79	18 ± 1.4	13.6 ± 0.240
Proline	4.7 ± 0.077	4.3 ± 0.38	4.22 ± 0.0865
Cysteine	2.0 ± 0.054	1.2 ± 0.11	1.12 ± 0.0700
Aspartic acid, asparagine	10 ± 0.091	12 ± 0.95	9.02 ± 0.129
Hydroxyproline	0.45 ± 0.056	0.42 ± 0.12	Not measured

To assess statistical differences, groups were compared using ANOVA followed by a post-hoc Tukey test for significance. Amino acids that were statistically significantly different between cultured quail and either conventional Australian *Coturnix* or Singapore *Coturnix* ($p < 0.05$) include histidine, lysine, methionine, phenylalanine, tryptophan, valine, serine, glycine, alanine, tyrosine, glutamic acid, and cysteine. Levels of isoleucine, leucine, threonine, arginine, proline, aspartic acid, and hydroxyproline are not significantly different than the levels in conventional quail. To further evaluate biological relevance of the amino acids with differences found to be

statistically significant, an assessment was performed to determine whether the measurements of these amino acids are below or above the comparator ranges by 10% or more (Table C.6.2.3-2). This is the approach taken by the US Food and Drug Administration (FDA) in assessing whether there is biological significance beyond statistical significance for animal clones such as the genetically engineered AquAdvantage Salmon (FDA 2008, FDA 2015).

Table C.6.2.3-2 Comparison of amino acid content in cultured quail to conventional quail (g/100g protein)

	Au Coturnix			**Sg Coturnix**			**Range in conventional quail**		**Exceedance range 10%**		**Cultured quail**
	Sample 1	*Sample 2*	*Sample 3*	*Sample 1*	*Sample 2*	*Sample 3*	*min*	*max*	*min*	*max*	*Measured (average)*
Histidine	3.3	3.1	3.3	2.60	2.66	2.66	2.29	5.11	2.06	5.62	4.2
Lysine	13	11	13	8.67	8.08	7.99	8.37	11.9	7.53	13.1	8.5
Methionine	3.5	3.2	3.1	2.64	2.67	2.69	2.28	6.79	2.05	7.47	2.3
Phenylalanine	5.0	4.3	4.3	3.65	3.77	3.81	3.93	7.01	3.54	7.71	4.8
Tryptophan	0.93	1.0	0.82		Not measured		0.834	1.74	0.751	1.91	1.6
Valine	6.4	5.2	5.3	4.49	4.65	4.62	5.03	6.13	4.53	6.74	5.7
Serine	4.9	4.2	4.4	3.72	3.80	3.87	2.31	4.87	2.08	5.36	5.8
Glycine	5.8	4.7	5.4	4.18	4.33	4.29	3.50	7.86	3.15	8.65	5.6
Alanine	7.6	6.3	6.6	5.05	5.39	5.27	2.89	6.47	2.60	7.12	5.6
Tyrosine	4.2	3.6	3.6	3.22	3.28	3.37	2.75	4.65	2.48	5.12	4.1
Glutamic acid, glutamine	20	17	17	13.5	13.5	13.9	1.27	14.3	1.41	15.7	15
Cysteine	1.0	1.2	1.3	1.04	1.16	1.16	0.876	8.90	0.788	9.79	2.0

Serine

Using a 10% exceedance of conventional ranges, serine exceeds the highest value (Table C.6.2.3-2).

Serine is a non-essential amino acid that is necessary for the metabolism of fats, fatty acids, and cell membranes as well as helping with muscle growth and the immune system. Here the No Observed Adverse Effect level (NOAEL) is used as a threshold, and a Margin of Exposure (MOE) approach is used where the ratio is calculated by dividing the NOAEL by the calculated maximum estimated human intake level. The average weight of a female Australian (71.1 kg) is used to calculate the MOE (Australia Bureau of Statistics 2017-2018).

The NOAEL for serine is above 12 g/day, or approximately 169.3 mg/kg/day in humans (the maximum amount administered in the study) (Miura *et al.*, 2021).

As shown in Table C.6.2.3-3 using conservative assumptions, taking the highest measured value in cultured quail, assuming that a 300 g serving contains 12% protein (36 g), the Margin of Exposure (MOE) to serine is 6 times lower than the NOAEL for serine. Therefore, slightly increased serine in cultured quail is well below effect levels and not expected to pose a food consumption hazard.

Table C.6.2.3-3. Estimation of daily intake for amino acids

	Average measurement in cultured quail (g/100 g protein)	**Amount in one 300 g serving (12% protein) (mg)**	**Estimated intake, based on average adult weight of 71.1 kg (mg/kg bw/day)**	**NOAEL or LOAEL (mg/kg bw/day)**	**Margin of Exposure**
Serine	5.8	2088	29.4	169.3	6

C.6.1.4 Minerals

Minerals are inorganic elements that help with structural functions involving skeletal structure and soft tissues as well as regulatory functions such as neuromuscular transmission, blood clotting, oxygen transport, and enzymatic activity. They are present in all meat and cell cultures. The mineral testing results are summarised in Table C.6.2.4-1 and provided in Appendix 21.

Table C.6.2.4-1 Mineral results (mg/kg)

	Cultured quail	**Conventional quail**			**Reported values**
		Au *Coturnix*	Au *Colinus*	Sg *Coturnix*	Minimum – Maximum
Calcium	11 ± 1	467 ± 244	91 ± 7	431 ± 381	60 – 291
Chromium	<0.05	0.13 ± 0.19	<0.050	<0.025	No data
Copper	1.3 ± 0.5	1.1 ± 0.0	1.5 ± 0.6	1.1 ± 0.1	1 – 7.65
Iodine	0.015 ± 0.01	0.009 ± 0.006	0.018 ± 0.018	0.3 ± 0.1	0.003 – 0.015
Iron	5.7 ± 0.5	13.7 ± 2.9	19.5 ± 6.4	15.4 ± 1.0	13 – 50.35
Magnesium	140 ± 17	167 ± 7	270 ± 28	239 ± 19	59.95 – 405.4
Manganese	0.21 ± 0.01	0.19 ± 0.01	0.21 ± 0.028	0.212 ± 0.003	0.18 – 0.82
Phosphorus	2327 ± 206	2110 ± 130	2370 ± 170	2070 ± 113	1000 – 3293
Potassium	4080 ± 234	2887 ± 80	3270 ± 438	2557 ± 127	1870 – 5798
Selenium	0.23 ± 0.03	0.15 ± 0.01	0.28 ± 0.05	0.16 ± 0.003	0.1432 – 0.276
Sodium	1187 ± 192	503 ± 31	510 ± 113	379 ± 27	34.65 – 740
Sulfur	1077 ± 76	2073 ± 55	2315 ± 40	Not measured	1200 – 7037
Zinc	13 ± 2	8.4 ± 0.2	7.5 ± 1.13	8.8 ± 0.5	7.4 – 27

Minerals lower than conventional range

Minerals below the measured range in conventional quail include calcium, iron, and sulfur. Cultured quail is therefore not considered a dietary source of these minerals, nor will be marketed as such. Vow cultured quail will be served to patrons in restaurants at limited serving sizes. It is not targeted to replace conventional quail in diets, but rather serve as a new food available to consumers, and therefore it is not anticipated to adversely affect the nutritional intake of consumers.

Margin of Exposure analysis for minerals exceeding levels in conventional quail

Potassium, sodium, and zinc levels were elevated in comparison to conventional quail. A comparison to recommended daily intakes and a Margin of Exposure (MOE) assessment was performed to evaluate whether any of these minerals could pose a health hazard from consuming Vow cultured quail. Where possible, conservative MOE calculation is reported as the lower bound of the Upper Level (UL) of intake divided by the highest measured value in Vow cultured quail. The UL is the maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans. The Adequate Intake (AI), Recommended Dietary Intake (RDI) or Suggested Dietary Target (SDT) values are provided for reference, where available (FAO/WHO 2004, National Health and Medical Research Council, 2017). The analysis is summarised in Table C.6.2.4-2 for Margin of Exposure assessments.

Potassium

Vow culture quail contains an average of 4080 mg/kg potassium. This level of potassium corresponds with the levels reported for 'Quail, lean flesh & skin, raw" in the Australian Food Composition Database, at 5000 mg/kg. No ULs have been established for potassium from dietary sources, and the AI is 2800 mg/day for adult females and 3800 mg/day for adult males (National Health and Medical Research Council, 2017).

The average amount of potassium in one 300 g cultured quail serving is 1224 mg. The level of potassium in cultured quail does not pose a food consumption hazard, and one serving of cultured quail can provide 32 – 44% of the AI levels of potassium for healthy adults.

Sodium

Vow culture quail contains an average of 1187 mg/kg sodium. The National Health and Medical Research Council recommended that adult population sodium intake levels should be reduced from the current average of about 3600 mg/day to a Suggested Dietary Target (SDT) of 2000 mg/day. The UL for sodium is 2300 mg/day for 14-18 year-olds (a UL was not determined for adults by the National Health and Medical Research Council, 2017).

The average amount of sodium in one 300 g cultured quail serving is 356 mg. The amount of sodium in one serving of cultured quail is approximately 18% of the SDT, and the MOE is 6 times below the UL. Therefore, the level of sodium in cultured quail does not pose a food

consumption hazard, nor is cultured quail anticipated to contribute to intake of sodium that would exceed the SDT.

Zinc

Vow culture quail contains an average of 13 mg/kg zinc. The RDI is 8 mg/day for adult females and 14 mg/day for adult males, and the UL for zinc is 40 mg/day (National Health and Medical Research Council, 2017).

The average amount of zinc in one 300 g cultured quail serving is 3.9 mg. The amount of zinc in one serving of cultured quail is approximately 29 – 49% of the RDI, and the MOE is 10 times below the UL. Therefore, the level of zinc in cultured quail does not pose a food consumption hazard, nor is cultured quail anticipated to contribute to intake of zinc that would exceed the RDI.

Table C.6.2.4-2. Margin of Exposure assessments for minerals exceeding comparator groups

	Average amount in cultured quail (mg/kg)	Amount mineral in one 300 g serving (mg/300 g)	Recommended intake (mg/day)[1]	Upper level of intake [UL] (mg/day)*	Margin of Exposure
Potassium	4080	1224	AI: 2800-3800	None established	N/A
Sodium	1187	356	SDT: 2000	2300**	6
Zinc	13	3.9	RDI: 8-14	40	10

N/A = not applicable
**For adults, unless otherwise noted **For 14-18 year olds*
[1]National Health and Medical Research Council (2017)

C.6.2.5 Vitamins

Vitamins are a group of substances needed for normal cell function, growth, and development. The amounts of vitamins in Vow cultured quail are compared to those measured in conventional quail, and of those reported in databases and in literature (Table C.6.2.5-1). The vitamin testing results are provided in Appendix 21.

Table C.6.2.5-1 Vitamin results (mg/100g)

	Cultured quail	**Conventional quail**			**Reported values**
		Au *Coturnix*	Au *Colinus*	Sg *Coturnix*	Minimum – Maximum
A (retinol)	<0.005	0.0227 ± 0.0127	0.0141 ± 0.007	0.05 ± 0.0011	0.01 – 0.07
B1 (thiamin)	0.50 ± 0.036	0.08 ± 0.02	0.14 ± 0.04	Not measured	0.05 – 0.28
B2 (riboflavin)	0.28 ± 0.10	0.5 ± 0.07	0.58 ± 0.18	0.41 ± 0.02	0.16 – 0.51
B6 (pyridoxine)	0.67 ± 0.061	0.44 ± 0.178	0.62 ± 0.04	0.476 ± 0.062	0.52 – 0.68
B7 (biotin)	0.10 ± 0.00	0.0012 ± 0.0002	0.00084 ± 0.00051	0.0175 ± 0.0012	No reports
B9 (folates)	0.268 ± 0.0150	0.0339 ± 0.00367	0.0309 ± 0.0023	0.0233 ± 0.00252	0.004 – 0.009
B12 (cobalamin)	0.619 ± 0.0104	0.00214 ± 0.00065	0.00183 ± 0.00074	0.00204 ± 0.00019	0.00043 – 0.0012
C (ascorbic acid)	<1	<1	<1	3.08 ± 0.33	5.1 – 7.2

Assessment of vitamins exceeding levels in conventional quail

Vitamins B1 (thiamin), B7 (biotin), B9 (folates), and B12 (cobalamin) were elevated in comparison to conventional quail. The Recommended Dietary Intake (RDI) values or Adequate Intake (AI) values are provided for reference (National Health and Medical Research Council, 2017, FAO/WHO 2004). There are no upper levels of intake (UL) for these vitamins, with the exception of vitamin B9 (Table C.6.2.5-2).

The following safety conclusions on these vitamins were made as a result of a Joint FAO/WHO Expert Consultation (FAO/WHO 2004): 1) Thiamin [vitamin B1] toxicity is not a problem because renal clearance of the vitamin is rapid; 2) Biotin [vitamin B7] toxicity is not a problem because of the limited intestinal absorption of biotin; 3) There is no evidence to suggest that it is possible to consume sufficient natural folate to pose a risk of toxicity; and, 4) Intake of 1000 mg vitamin B12 has never been reported to have any side-effects.

It is noted that Vitamin B7 (biotin), vitamin B9 (folates) and Vitamin B12 (cobalamin) in Vow cultured quail exceed the RDI. However, vitamins are listed in the Australia New Zealand Food Standards Code as permitted food additives (Schedule 16), vitamins (Schedule 17), and/or permitted processing aids (Schedule 18). Furthermore, the intake of vitamin B7 has been shown to be beneficial because it promotes healthy skin and the growth of hair and nails (Patel *et al.* 2017). Additionally, a deficiency in biotin levels has been connected to erythematous and seborrheic type dermatitis along with conjunctivitis, alopecia, and even central nervous systems abnormalities such as paresthesia of extremities (FAO/WHO 2004). Folate deficiency is one of the most common vitamin deficiencies in Australia (Healthdirect Australia 2020a). Some conventional foods such as liver contain 1.450 mg/kg folate (FSANZ Australian Food Composition Database, 2022). Vitamin B12 (cobalamin) is a necessary vitamin to support the normal function and development of nerve cells (Troen 2012). Deficiencies in vitamin B12 are linked to pernicious anemia and hypochlorhydria from atrophic gastritis (FAO/WHO 2004). Additionally, vitamin B12 has been suggested to help reduce the risks of certain cancers such as cervical cancer (Ryan-Harshman and Aldoori 2008). Australian Dietary Guidelines (Healthdirect Australia 2022) recommend people with a vegan diet take a B12 supplement, as deficiency is more common in this group of people. Since there is no toxicity associated with consuming these levels of vitamins, and consumption of cultured quail will be limited by serving size and availability at restaurants, presence of these vitamins in cultured quail is not anticipated to pose a consumption hazard.

Table C.6.2.5-2: Margin of Exposure assessments for vitamins

	Average measurement in cultured quail (mg/100g)	Amount vitamin in one 300 g serving (mg)	Recommended dietary intake [RDI] or Adequate intake (AI) (mg/day)*	Upper level of intake [UL] (mg/day)*	Margin of Exposure
Vitamin B1 (thiamin)	0.50	1.5	1.1 (RDI)	No UL	N/A
Vitamin B7 (biotin)	0.087	0.261	0.025 (AI)	No UL	N/A
Vitamin B9 (folates)	0.268	0.804	0.4 (RDI)	1.7 (as folate, equivalent to 1 mg/day folic acid)	2.1
Vitamin B12 (cobalamin)	0.619	1.86	0.0024 (RDI)	No UL	N/A

*FAO/WHO 2004, National Health and Medical Research Council, 2017

C.6.3.6 Fatty acids

According to proximate analysis, Vow cultured quail contains 1.5 ± 0.1 g/100 g fat. The low levels of fat are expected for a product composed of fibroblast cells. Of this, 42.1% are saturated fats, 54.3% are monosaturated fats, 0.9% are polyunsaturated, 0.5% are monotrans fats, and 2.2% are poly trans fats. Vow cultured quail is composed of slightly higher ratios of saturated fats, monounsaturated fats, monotrans fats, and polytrans fats than conventional quail. Vow cultured quail contains a lower ratio of polyunsaturated fats than conventional quail. The fatty acid testing results are provided in Appendix 21.

However, due to the lower overall fat content, there are lower levels of these fats overall in Vow cultured quail. One 300 g serving of Vow cultured quail is only expected to contain a maximum of 9 g of fat (based on 3%, or 3 g/100 g Vow cultured quail). Given that the average fat intake of Australian adults is 61.0-79.4 g/day[1] (Australian Bureau of Statistics 2011), a serving of cultured quail will not contribute significantly to fat intake. Recommendations suggest limiting intake of saturated and trans fats to less than 8-10% of total energy intake (National Health and Medical Research Council 2017). As each gram of fat yields approximately 38 kilojoules, total kilojoules from fat in one 300 g serving is 342 kJ. Saturated and trans fats make up 44.8% of the total fat, equal to 153 kJ per serving. The recommended daily intake of energy for Australian adults is 8700 kJ, making the recommended intake for energy from fats (8%) 696 kJ (Healthdirect 2020b). The energy from saturated and trans fat (153 kJ) is ~4.5 times lower than the 696 kJ conservative recommended daily intake of saturated and trans fat. Therefore, a serving of cultured quail will not contribute to negative health outcomes associated with high intake of saturated and trans fats.

[1] The reported average daily intake from fat is 31% in Australia (Australian Bureau of Statistics , 2011). For males, the average energy intake is 9,655 kilojoules (kJ) so 0.31 X 9655 = 2993 kJ / 4.184 kcal/kJ = 715 calories / 9 cal/g = 79.4 g/d. For females, the average energy intake is 7,402 kJ , equivalent to 61 g/d.

Table C.6.2.6-1 Fatty acid results (%)

	Cultured quail	**Conventional quail**	
		Au *Coturnix*	Sg *Coturnix*
Total saturated	42.1 ± 1.8	30.1 ± 0.3	27.4 ± 1.0
Total monounsaturated	54.3 ± 1.8	49.4 ± 2.6	42.1 ± 1.5
Total polyunsaturated	0.9 ± 0.3	20.5 ± 2.7	25.7 ± 2.2
Total mono trans	0.5 ± 0.1	0.1 ± 0.087	0.40 ± 0.029
Total poly trans	2.2 ± 0.2	<0.1*	

*Below limit of detection, <0.1 %

C.6.3 Information on the toxicity of the novel food ingredient derived from the new source

The reagents and processing aids used in the manufacture of Vow cultured quail include basal and growth media, media additives, cryoprotectant, antifoaming agent, and cleaning agents. A risk analysis of the inputs is performed for each input, including a review of its source and Certificate of Analysis from the supplier, regulatory analysis, an *in silico* analysis using Toxtree to assess the potential for human health hazard (where appropriate), and calculation of Margin of Exposure (MOE) based on conservative exposure scenarios. For any substances with a MOE < 100, measurement of the residue in the final cultured quail product was performed. Each of the substances measured in the final cultured quail product resulted in an MOE far exceeding the threshold of 100 (MOEs ranged from four to six orders of magnitude for measured substances). The analysis demonstrates that no inputs pose a food safety consumption hazard.

Certificates of Analysis for each reagent and processing aid are provided in Appendix 24. A list of media components, their concentrations, details of the risk assessments and measurements from final product testing can be found in Appendix 25.

In addition, a comparative analysis is performed of cultured quail, working cell banks, and conventional quail genomes to evaluate potential for genetic drift and production of substances that could be potentially hazardous to human health. It is concluded from the analysis, found in Appendix 29, that there is minimal genetic drift in the cultured quail cells, and no mutations in sequences that could result in production of harmful food substances.

C.6.3.1 Risk assessment of reagents and processing aids

All reagents and processing aids were evaluated for safety. Substances that are authorised food additives as listed in the Australia New Zealand Food Standards Code (‘the Code’) are considered safe for consumption. For any substance not already listed as an authorised food additive in the Code, a safety assessment is performed to evaluate the potential human health risk in the context of oral consumption of Vow’s cultured quail, by assessing the potential for exposure and comparing expected levels with levels of the substance in conventional foods, and

with the No Observed Adverse Effect Levels (NOAELs). Appendix 25 contains the detailed safety analysis of components.

First, where relevant, an assessment using Toxtree, an online open-source application that performs *in silico* analysis of chemicals based on their structure and assesses health hazards by applying a decision tree approach. The program classifies chemicals according to the Cramer rules, the Benigni/Bossa rules for carcinogenicity and mutagenicity, and the ILSI/Kroes decision tree for the Threshold of Toxicological Concern (TTC).

In addition to this *in silico* analysis, a Margin of Exposure (MOE) is calculated based on the most conservative scenario, assuming that all of the substance accumulates in the cells and is present at maximum concentration in the final product. No Observed Adverse Effect levels (NOAELs) are used as thresholds, and a MOE approach is used where the ratio is calculated by dividing the NOAEL by the calculated maximum estimated human intake level. The average weight of a female Australian (71.1 kg) is used to calculate the MOE (Australia Bureau of Statistics 2017-2018).

The MOE analysis is intended to evaluate the potential risk using the most conservative scenario. Realistically, most inputs are not expected to accumulate, and many will degrade or be metabolised during cell growth. For substances that are not genotoxic nor carcinogenic, a MOE ≥ 100 is considered of low concern. None of the substances evaluated are genotoxic or carcinogenic; therefore, for any substances with a MOE <100, analytical measurement of that residue in the final cultured quail product was performed, followed by a risk assessment based on the residual concentration.

Methods appropriate for the sensitivity required to detect each substance were used to measure the residues in the final product. Quail cells from the final product were lysed and substances extracted according to Folch extraction methods, then detection *via* Ultrahigh Performance Liquid Chromatography - Electrospray Ionisation Ion Trap tandem Mass Spectrometry (UPLC-ESI-MS/MS). Standards were used to determine the limits of detection and quantification, and to validate the methods. Details of the methods and results are provided in Appendix 26.

Table C.6.3.1-1 Overview of reagents and processing aids

Substance	Regulatory status
Vow basal media	See section C.6.3.1.1 for analysis of components
Supplier basal media	A risk assessment of Supplier basal media has been provided by the supplier directly to FSANZ
Media additives	See section C.6.3.1.2 for analysis of components
Cryoprotectant	The substance is diluted throughout the process, is not toxic, and does not pose a food safety risk, as described in C.6.3.1.3.
Antifoam agent	Permitted food additive in Australia New Zealand Food Standards Code Schedules 15 and 16 and as a processing aid in Schedule 18 See section C.6.3.1.4 for further analysis
Cleaning agents	Main component of one (potassium carbonate) is on the Australia New Zealand Food Standards Code Schedule 15 and 16. Main components of another are citric acid and hydrogen peroxide. See section C.6.3.1.5 for further analysis.

C.6.3.1.1 Basal media

The basal media consists of cell culture media provided by a third party, combined with other common cell culture media components like amino acids, sugars, and salts. Basal media components support the growth of rapidly dividing cells that require large amounts of proteins and nucleic acids, fuel the cells, and buffer pH. Appendix 25 contains the detailed safety analysis of components provided by a third-party supplier. Appendix 24 contains the Certificates of Analysis for all Vow components.

Amino acids are measured in the final product. For amino acids added by Vow to the basal media, final levels are comparable to those found in conventional quail. Sugars and salts added into the basal media by Vow have long histories of safe use in food.

C.6.3.1.2 Media additives

Media additives provide the necessary factors to support cell viability and mimic a natural physiological environment. Vow's media additives include additional amino acids, polyamine compounds, growth factors, and other additives. All of the additives have a MOE > 100, and are already naturally found in food or are present in the human body. Therefore, use of these additives do not pose a food safety risk in cultured quail.

Growth factors

Two recombinant growth factors are added to media during production to support the growth of the quail cells. The amount of these growth factors in the final Vow cultured quail was measured in at least three independent batches using ELISA and Western Blots.

One recombinant growth factor (referred to as Growth Factor #1) is sourced from barley seed and contain the purified recombinant growth factor along with selected barley seed proteins. The amount of the recombinant growth factor is typically in the range of 20-30% of the total purified protein content. The barley seed proteins serve as stabilizing proteins that can prolong the lifetime of the recombinant protein and may enhance the bioactivity of growth factors in standardised bioassays. There is no carryover of barley or gluten into the final product, as demonstrated in the allergenicity testing of the final product (Section C.6.1.4). The growth factors are free of endotoxins. The amount of GMO-DNA is measured by the supplier, and is below <1 pg/µg growth factor; therefore, no GMO-DNA from barley is present in Vow cultured quail.

For the second, Vow may interchangeably use one of two similar recombinant growth factors:

- One of these (referred to as Growth Factor #2a) has a similar manufacturing profile to the first growth factor. It is sourced from barley and the media additive contains the purified recombinant growth factor along with select barley seed proteins. The amount of the recombinant growth factor is in the range of 20-30% of the total purified protein content.
- The second (referred to as Growth Factor #2b) is sourced from an *Escherichia coli* (*E. coli*) BL21 (DE3) strain and contains only the purified recombinant growth factor. The amount of the recombinant growth factor is >95% of the total purified protein content, where the remaining <5% is due to agglomeration of the recombinant growth factor. The endotoxin levels were not detected in the growth factor at < 0.2 EU/µg of protein, evaluated by gel clotting method.

The results originally submitted in the Vow Cultured Quail Novel Food Dossier were for cultured quail manufactured with a combination of Growth Factor #1 and Growth Factor #2a. Vow has retested the suite of parameters of the final product to evaluate any potential effects on food safety when Vow cultured quail is manufactured with the combination of Growth Factor #1 and Growth Factor #2b. A full description of Growth Factor #2b and detailed safety assessments associated with manufacturing Vow cultured quail with Growth Factor #2b are summarized in the Supplement, “FSANZ Supplemental Update”. No food safety concerns are associated with the use of Growth Factor #2b.

The Certificates of Analysis and Background documents are contained in Appendix 27.

Growth factor safety was evaluated using a weight-of-evidence approach, using measured levels present in Vow cultured quail; comparison to levels found in other foods including conventional

quail, conventional chicken, and conventional beef; comparison to levels naturally found in human bodies; studies on genotoxicity, mutagenicity, or cytotoxicity of the growth factors; studies on degradation in the gastrointestinal tract; and studies of bioactivity of the growth factors. The analyses of the growth factors used to manufacture Vow cultured quail provide evidence that the growth factors are similar to those present in conventional meat, are present at low levels, and degrade with cooking and digestion. Therefore, use of these growth factors in the manufacture of Vow cultured quail are concluded to not pose a food safety hazard.

ELISA tests were used to measure levels of each growth factor found in the final product. In addition, Jess Simple Western ('Jess'), a highly sensitive multiplexed Western blot, was used to quantify and verify one of the growth factors in Vow cultured quail samples. Full results can be found in Appendix 28.

Risk assessment of Vow cultured quail Growth Factor #1

Growth Factor #1 (porcine) was measured in Vow cultured quail using three different approaches: ELISA, Jess Simple Western (a highly sensitive multiplexed Western Blot), and a Western blot analysis. The levels of the growth factor in one serving of Vow cultured quail are within the range of the same type of growth factor consumed in one cup of milk. Further, the amount of growth factor is 13 - 41 times lower than amounts administered without adverse effects in human subacute and subchronic studies. The results demonstrate that the growth factor is present at levels comparable to those in conventional food; further degradation would be anticipated with cooking as well as proteolytic degradation in the gastrointestinal tract. The growth factor levels measured in cultured quail are well below the safe levels of the growth factor orally administered in human studies. Therefore, use of the growth factor in the manufacture of cultured quail is concluded to not pose a food safety hazard.

Risk assessment of Vow cultured quail Growth Factor #2a

Growth factor #2a (bovine/porcine, derived from barley) was measured using an ELISA test kit. Measurements of the growth factor in uncooked Vow cultured quail, cooked Vow cultured quail, conventional quail, and chicken were performed. Residue measurement demonstrates that only 24.5% of the total amount of growth factor added during the whole manufacturing process is present in the final uncooked product. Residue measurement in uncooked versus cooked quail demonstrates that cooking degrades the growth factor further, reducing its levels by 79.5%. Uncooked cultured quail has approximately 60% of the amount of the growth factor as compared to conventional quail, and 71% as compared to conventional chicken. When compared to levels produced in the human body, the levels present in cooked quail are equivalent to consuming 0.5% of the amount of the growth factor produced daily in adults. The growth factor levels in cultured quail are below the levels measured in conventional quail and chicken, are degraded with cooking, and represent only a small fraction of endogenous production in adults. Therefore, use of the growth factor #2a in the manufacture of cultured quail is concluded to not pose a food safety hazard.

Risk assessment of Vow cultured quail Growth Factor #2b

Growth factor #2b (bovine, derived from *E. coli*) was measured using an ELISA test kit. Measurements of the growth factor in uncooked Vow cultured quail, cooked Vow cultured quail, conventional quail, and beef were performed. Residue measurements demonstrate that < 1.5% of the total amount of growth factor added during the whole manufacturing process is present in the final uncooked product. Experimental results demonstrate that cooking degrades the growth factor further, reducing its levels to below the lowest level of quantification (LLOQ). When compared to levels produced in the human body, the levels present in uncooked quail are equivalent to consuming <1.5% of the amount of the growth factor produced daily in adults. The growth factor levels in cultured quail are below the levels measured in conventional quail and beef, are likely degraded with cooking, and represent only a small fraction of endogenous production in adults. Therefore, use of Growth Factor #2b in the manufacture of cultured quail is concluded to not pose a food safety hazard.

C.6.3.1.3 Cryoprotectant

A cryoprotectant is used in the cell banking process. Given the dilution of the substance throughout the seed train process, the substance would only be present in the final product at insignificant levels and does not pose a food safety hazard.

C.6.3.1.4 Antifoam agent

An antifoaming agent is used to regulate headspace foaming in mammalian cell culture bioreactors. The Australia New Zealand Food Standards Code lists the agent as a permitted food additive in Schedules 15 and 16 and as a processing aid in Schedule 18. The levels of the antifoaming agent used are also below limits for non-standard food in the US.

Under the most conservative scenario where all of the substance accumulates in the cells and is present at maximum concentration in the final product, the MOE is estimated to be well above 100, so its use does not pose a food safety hazard.

C.6.3.1.5 Cleaning agent

Cleaning agents are used to clean equipment used in the process. All cleaning agents have little toxicity, are intended for cleaning of food-processing equipment, are used in compliance with the instructions provided by the manufacturer, and are not expected to be present in the final product as equipment is rinsed after use.

C.6.3.2 Genetic stability

Animals, including avian species such as quail, generally have not evolved the ability to produce toxins. The ability to express toxic substances and anti-nutrients is generally limited to microorganisms and plants, and in some animal species evolved to produce venomous toxins. However, a comprehensive literature review and search on the ‘Animal toxin annotation project’

on UniProt demonstrates that there are no known allergens, toxins, or anti-nutrients produced by quail.

Therefore, the capacity to produce toxins is considered to be minimal in quail cells. Regardless, whole genome sequencing (WGS) was performed to investigate the potential for genetic differences between conventional quail (*Coturnix japonica)*, Vow working cell bank cells (vWCB), and the final cultured quail cells. The sequenced genome was analysed to determine the number of DNA variants (single nucleotide variants –SNVs) in each sample, then cross-examined with conventional vs experimental variants, to isolate mutations that may have arisen through culture, and to evaluate whether these changes may result in increased food safety risks. Given a lack of known allergens, anti-nutrients, and toxins in quail meat, modified genes were assessed for potential for novel production of toxins, anti-nutrients, and allergens. In addition, an assessment of SNVs that could result in radical changes to the primary tissue resulting in malignant tumours were evaluated, which would result in distinct Gene Ontological enrichments, which have been highly informed by human and mouse cancer experiments (and thus heavily biased toward positive detection of such an event).

The full genetic stability report is provided in Appendix 29.

Summary of Results

Within-sample group consistency is high. Using the same indicators at group level shows that 97.2% of observed variants arose between conventional quail to Vow working cell bank cells (vWCB), and remain concordant in the vCQ final product. Almost all genetic variation observed was found to be generated in the transition from conventional quail to vWCB, with only 0.9% of all observed variants arising during the transition from vWCB to cultured quail final product.

Genetic instability in cell lines can be a result of variations leading to genetic damage, or inability to replicate or repair DNA. For the identified variants in all genetic stability experiments, there was no sign of significant genetic damage to DNA replication or repair pathways, indicating a genetically stable outcome. No gene ontological (GO) terms associated with cancer development were observed.

Conclusion

There are no identified variants in cultured quail cells predicted to result in production of toxins, anti-nutrients, allergens, or other substances of concern. It is concluded that there is minimal genetic drift in the cultured quail cells, and no mutations in sequences resulting in production of harmful food substances.

C.6.4 Safety assessment reports prepared by international agencies or other national government agencies

As of January 2023, only two countries (Singapore and the US) have made public regulatory announcements indicating the approval for sale of cultured meat products. Vow cultured quail has not yet been reviewed by international agencies or other national government agencies, but is currently under review by the Singapore Food Agency.

D. Information on dietary exposure to the novel food

D.1 A list of the foods or food groups proposed to or which might contain the novel food ingredient or substance

Vow cultured quail is intended to be used as an ingredient in a meat-based product. The cultured quail can be mixed with existing authorised food ingredients, and will be supplied to customers as frozen products, *e.g.,* pre-shaped 2 kg roll of meat that can be cut into medallions.

For example, Vow cultured quail may be mixed with calcium chloride, microbial transglutaminase, oil, textured vegetable protein, oils or fats, or other authorised food ingredients. Vow cultured quail is intended to make up the bulk of the product.

D.2 The proposed level of the novel food ingredient or substance for each food or food group

Vow cultured quail is intended to be used at a maximum of 300 g of cultured quail per dish, mixed with other authorised food ingredients. Initially, the final food product will be distributed through restaurants. Chefs will be provided with preparation instructions, including minimum cooking temperature.

D.3 For foods or food groups not currently listed in the most recent Australian or New Zealand (NNSs), information on the likely level of consumption

One serving size, or the Estimated Daily Intake (EDI) of cultured quail is 300 g/day. A conservative average-intake consumer is calculated by assuming a person eats cultured quail once a week or 52 times a year, leading to an annual quail consumption of 15,600 grams, equivalent to 43 g/day. A high-intake consumer is calculated by assuming a person eats cultured quail 3 times a week or 156 times a year, leading to an annual quail consumption of 46,800 grams, equivalent to 128 g/day.

The EDI for cultured quail meat can be compared to the consumption of conventional quail meat in Australia. While there are no direct figures, the estimated number of quail produced in 2003 was 6.5 million annually (Scolexia Animal and Avian Health Consultancy, 2009). Recently, it was reported that 3 million ‘other’ poultry including geese, turkeys, quail and ducks are raised in Australia (Australian Bureau of Statistics, 2022). Using an assumption that quail makes up one-quarter of ‘other’ poultry, 750,000 quail are produced annually.

According to unverified sources (purveyors), the 5th percentile weight of meat on a quail is 160 g, and the 95th percentile is 200 g. Assuming 200 g of meat on one quail, the annual estimated consumption of conventional quail in Australia based on recent official reports is 750000 quail * 200 g = 150 tonnes of quail, and up to 1300 tonnes based on the 2003 report (6.5 million quail *200 g).

Assuming 10% of the Australian population (25.4 million people) consumes quail, consumption of 150 – 1300 tonnes/year translates to 6 - 51 g of quail consumed per person per year in Australia. However, it is more likely that quail is consumed by a smaller subpopulation at a higher rate. Therefore, a more realistic calculation considers a smaller subpopulation of adult consumers who would consume quail on a monthly or weekly basis. A consumer eating one quail per month, or 200 g/month, would consume 2400 g per year, and a consumer eating one quail per week, or 200 g/week consumes 10,200 g per year of conventional quail.

This value is similar to the conservative average-intake consumer consumption of 14,820 g cultured quail per year. For high-intake consumers estimated to consume up to 46,800 g of cultured quail, numbers are more comparable to consumption of chicken. The average person in Australia and New Zealand consumes 41-49 kg of chicken per year (OECD 2022, Australian Chicken Meat Federation 2022). While there are no data available on the amount of quail consumed in restaurants per year, poultry holds a 45% share of the food service demand in dining out establishments (Thomas Elder Markets, 2021).

D.4 The percentage of the food group in which the novel food ingredient is proposed to be used or the percentage of the market likely to use the novel food ingredient

Vow cultured quail will be served to patrons in restaurants at limited serving sizes. It is not targeted to replace conventional quail in diets, but rather serve as a new food available to consumers. Given the channels through which it will be sold, it is not anticipated to significantly replace any food group.

D.5 For foods where consumption has changed in recent years, information on likely current food consumption

There is no available information on consumption of cultured quail.

D.6 Data to show whether the food, or the food in which the novel food ingredient is used, is likely to replace another food from the diet, if applicable

Not applicable.

D.7 Information relating to the use of the novel food or novel food ingredient in other countries, if applicable

Not applicable.

E. Information on the nutritional and health impact of the novel food

E.1 Information to demonstrate that the use of the novel food or novel food ingredient will not cause a nutritional imbalance in the diet

The consumption of cultured quail is not anticipated to have any impact on nutrient availability or result in nutritional imbalance. Cultured quail will be available to patrons of restaurants at limited serving sizes; it is not anticipated to serve as a substantial substitute for any food group or type of protein. A comprehensive literature search was conducted and did not identify any published literature to suggest that consumption of cultured quail would be associated with nutritional imbalance in the diet. Appendix 16 contains details on conducting the literature search. In addition, a nutritional analysis of cultured quail concluded that amino acid, mineral, vitamin, and fatty acid content does not pose a safety risk. Section C.6.2 provides the detailed analyses.

E.2 Information to demonstrate that the addition of the novel food ingredient will not create a significant negative public health impact

Not applicable; the purpose for adding a novel food ingredient does not relate to a potential beneficial physiological or health-related outcome. A comprehensive literature search was conducted and did not identify any published literature to suggest that consumption of cultured quail would be associated with negative public health impact, nor is cultured quail associated with allergic, toxic, or adverse health effects. Appendix 16 provides details on the literature search.

F. Information related to potential impact on consumer understanding and behaviour

F.1 Information to demonstrate the level of consumer awareness and understanding of the novel food or novel food ingredient

Consumer awareness of cultured meat is generally low, mostly driven by the fact that it is largely unavailable to consumers at the moment. The first cultured meat product was approved for sale in Singapore in 2020 (Lucas, 2020). GOOD Meat (formerly Eat Just), who received the initial approval in Singapore and the US, as well as Upside in the US remain the only companies to offer cultured meat products for sale in the world as of October 2023.

A literature review was performed of studies related to consumer awareness of cultured meat in Australia. A very limited number of studies have specifically addressed the market in Australia and New Zealand. A majority of respondents to a survey of US and UK adults found that over half (59% of US respondents and 54% of UK respondents) had no prior familiarity with cultured meat. 82% of respondents in both the US and UK said they supported the technology after it was explained to them (Szejda et al., 2021). Consumer research suggests that animal welfare, environmental, and health concerns are primary considerations for deciding to try cultured meat (Bryant & Barnett, 2020).

Outside of commercial sales, SuperMeat, an Israel-based cultured meat company, began testing consumer acceptance of their cultured chicken product by offering it for free to the public in 2022. In initial samples, the company gave people an option to try two chicken burgers, one traditional and one cultured. Over 70 percent of consumers chose to try the cultured chicken. Currently, over 10,000 people from around the world are on a waiting list to visit the SuperMeat test kitchen in Tel Aviv, showing consumer interest in trying cultured meat (Bottinelli, 2022).

F.2 Information on the actual or potential behaviour of consumers in response to the novel food or novel food ingredient

A literature review was performed of studies related to consumer acceptance of cultured meat. A number of studies have been conducted globally to assess consumer attitudes around cultured meat. Although early studies on consumer attitudes revealed consumer concerns about the unknown, more recent studies show that consumer awareness of cultured meat and willingness to try it are increasing. Younger generations are more likely to want to try cultured meat and see it as a potential substitute for conventional meat (Bryant & Barnett, 2020).

In a recent study, 80% of adults in both the US and UK say they would be willing to try cultured meat (Szejda et al., 2021). A 2021 study of South African adults aged 18-61 found that 60% of adults were highly likely to try cultured meat, and 53% were likely to purchase it (Szejda et al., 2021). In a study of consumers across the US, India, and China, Bryant et al. (2019) find that across all three geographies those who consume meat were more likely to purchase cultured meat if available (compared to those following a vegetarian, vegan, or pescatarian diet). They also found that familiarity with cultured meat was associated with higher likelihood of purchasing cultured meat.

A multi-country study in 2018/2019 of consumers which included over 250 respondents from New Zealand looked at the willingness to try cultured meat and pay a premium for it. Characteristics of participants were tested as drivers or inhibiting factors of whether consumers would try cultured meat and pay a premium. The study found that “food neophobia, having food allergies, being a locavore, and having concerns about food technology were found to be inhibiting factors towards willingness to try, buy, and pay a price premium for cultured meat. Food curiosity, meat importance, and a consumer’s perception of cultured meat as a realistic alternative to regular meat were found to be important drivers that positively impacted

consumers' willingness to try, buy and pay more." Results were presented across all countries, without a break-out specifically for respondents from New Zealand (Rombach et al., 2022).

In a global survey of consumers in ten countries, Australian willingness to try cultured meat is similar to other countries like the US (Siegrist and Hartmann 2020). Forty-nine percent of Australian consumers surveyed would be willing to consume cultured meat (Garcez de Oliveira Padilha et al., 2021). In Sydney, a smaller study conducted in 2019 of Gen Z adults ages 18-24 found that 28% were prepared to try cultured meat (Bogueva and Marinova, 2020).

F.3 Information to demonstrate that the food(s) containing the novel food ingredient will not adversely affect any population groups (e.g. particular age or cultural groups)

Vow cultured quail, as demonstrated in Section C.6.2 of the safety dossier, resembles conventional quail. Poultry is prevalent in diets of Australians and New Zealanders of broad cultural groups and ages. It is not anticipated that the novel food will impact any other particular population groups beyond the populations that are already allergic to poultry products.

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