GOURMEY

Novel Food Application to Amend the Australia and New Zealand Food Standards Code for Cell Cultured Duck

Submitted by:
Suprême SAS (Gourmey)
La Pépinière Genopole Entreprises
4 Rue Pierre Fontaine
91058 Évry-Courcouronnes
France

Date of submission: 8th August, 2025

GOURMEY

Table of Contents

List of Tables/Figures

List of Appendices

GOURMEY

Appendix 22 Methods of Analysis
Appendix 23a Transcriptomic toxicity assessment [CCI]
Appendix 23b FR003143 suppl1 blastp swissprot toxin [CCI]
Appendix 23C FR003143 suppl2 blastp trembling toxin [CCI]
Appendix 24 Process parameters operational limits [CCI]
Appendix 25 Supplier statement on Absence of Human Proteins [CCI]

List of Abbreviations

APC = Aerobic Plate Count
APHIS = Animal and Plant Health Inspection Service
AI = Avian Influenza
AOAC = Association Of Official Analytical Collaboration
ASF = African Swine Fever
BOLD = Barcode of Life Data System
BSA = Bovine Serum Albumin
BSCT = Biosafety Cabinet
BSE = Bovine Spongiform Encephalopathy
BW = Body Weight
CDC = Centre for Disease Control and Prevention
CFR = Code of Federal Regulations
CoA = Certificate of Analysis
CSC = Cancer Stem Cells
CSF = Classical Swine Fever
dESC = duck Embryonic Stem Cells
DAO = Diamino Oxidase
DNA = Deoxyribonucleic Acid
DNMT-3 = DNA methyltransferase 3
DMSO = dimethyl sulfoxide
ECHA = European Chemicals Agency food contact materials (FCM).
ECDC = European Centre for Disease Prevention Control
ECSC = Embryonic Cancer Stem Cells
EFSA = European Food Safety Authority
EPA= Environmental Protection Agency
EOP = End of Production
FAO = Food and Agriculture Organization
FBS = Foetal Bovine Serum
FCC = Food Chemical Codex
FCM = Food Contact Materials
FDA = Food and Drug Administration
FMD = Food-to-Mouth Disease
FSANZ = Food Standards Australian New Zealand
FSIS = Food Safety Inspection Service
FSMA = Food Safety Modernization Act
GC/MS = Gas Chromatography coupled with Mass Spectrometry
GF = Growth Factor
GI = Gastrointestinal
GLP = Good Laboratory Practices
GMP = Good Manufacturing Practices
GRAS = Generally Recognized as Safe
HACCP = Hazard Analysis and Critical Control Points
HARPC = Hazard Analysis and Risk-based Preventive Controls
HPB = Health Promotion Board
HPAI = High Pathogenic Avian Influenza
HPP = High-Pressure Processing
HAS = Health Science Authority
ICH = International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IDE = Insulin-degrading Enzyme
IgE = Immunoglobulin E
MCB = Master Cell Bank
NAD+ = Nicotinamide Adenine Dinucleotide
ND = Not Determined
NDV = Newcastle Disease Virus

NGS = Next Generation Sequencing
NOAEL = Non-observed Adverse Event Level
OCT4 = Octamer-binding transcription factor 4
OECD = Organization for Economic Co-operation and Development
LD50 = Lethal Dose 50
LDH = Lactase dehydrogenase
LPAI = Low Pathogenic Avian Influenza
LMWF = Low Molecular Weight Fractions
LSAS= Label Submission and Approval System
PBS = Phosphate-buffered saline
PCB = Polychlorinated biphenyl
PCR = Polymerase Chain Reaction
qPCR = real time PCR
RDA = Reference Daily Allowances
RNA = Ribonucleic Acid
RT-PCR = Reverse Transcription PCR
RT-qPCR = Reverse transcription-quantitative polymerase chain reaction
RIVM = Dutch National Institute for Environment Hygiene and Public Health (translation)
SOP = Standard Operating Procedure
PDT = Population Doubling Time
PEG = Polyethylene Glycol
PGG = Polypropylene Glycol
ppm = parts per million
Prp = Prion Protein
TDI = Tolerable Daily Intake
TERT = telomerase reverse transcriptase
TF = Transcription Factors
TVC = Total Viable Cells
UL = Tolerable Upper Intake Limit
USDA = United States Department of Agriculture
USP = United States Pharmacopeia
UV = Ultraviolet
WCB = Working Cell Bank
WHO = World Health Organization
WOIE = World Organization for Animal Health

GOURMEY

GENERAL REQUIREMENTS

This section is completed in accordance with Chapter 3.1.1 (General Requirements for Applications) of the Food Standards Australia New Zealand Application Handbook (FSANZ, 2024a).

1. Form of the application

The application is presented in English.

2. Applicant details

2.1. Applicant details (individual or organisation's) name

Suprême SAS (Gourmey)
La Pépinière Genopole Entreprises
4 Rue Pierre Fontaine
91058 Évry-Courcouronnes
France

2.2. Name of the contact person/s

[redacted]
Head of Regulatory Strategy
[redacted]

[redacted]
Head of Quality & Regulation
[redacted]

2.3. Nature of applicant's business

Suprême SAS (commercially known as Gourmey) is a cultured food company based in Paris, France. Gourmey utilizes an innovative production process to produce high-quality foods from real animal cells with an initial focus on premium products. Gourmey's flagship product, cell cultured duck is the subject of this application. Gourmey's novel food is intended to be used as an ingredient in cell cultured duck meat analogues such as cell cultured duck "foie gras" and cell cultured duck pâté.

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

Not applicable.

3. Purpose of the application

Suprême SAS (Gourmey) is submitting this application to amend the Australia New Zealand Food Standards Code (referred to as the "Code") to allow the use of cell cultured duck to be used as a food ingredient. The cell cultured duck will be mixed with other authorised ingredients to be used in duck meat analogues such as "cell cultured foie gras" and "cell cultured duck pâté".

Gourmey's cell cultured duck is considered to meet the definition of cell cultured food as outlined in draft Standard 1.5.4 Cell-cultured foods and meets the current definition of a "non-traditional food" as per Standard 1.5.1 of the code where cell cultured duck as a substance does not have a history of human consumption as a food in Australia or New Zealand. As such, cell cultured duck is considered to be a novel food as the ingredient has no previous history of human consumption in Australia or New Zealand along with the need to assess "the process by which the food has been prepared" and "the source from which it is derived." Whilst there is no history of consumption of cell cultured duck, duck meat is widely consumed globally. Gourmey's cell cultured duck is comparable to conventional duck and meat products in composition (see Section E for more information).

It is the view of Gourmey that the following Schedules will need to be amended to permit the use of cell cultured duck to be used as a food ingredient in food marketed in Australia and New Zealand:

- Schedule 3 - Identity and purity
- Schedule 25A - Permitted cell-cultured foods

4. Justification for the application

An application for regulatory approval is required for Gourmey's cell cultured duck as it is considered a non-traditional novel food requiring pre-market approval.

Gourmey's flagship product will be a cell cultured duck "foie gras", which offers consumers an alternative to conventionally produced foie gras. Foie gras production has been banned in Australia, and currently, only processed and shelf-stable forms are permitted for import as there are no biosecurity measures that apply to raw poultry (which includes duck) and even poultry pâté, pastes and livers that are cooked are deemed as risk foods and must undergo thermal processing to eliminate food-borne pathogens such as *Salmonella* spp., and *Listeria monocytogenes*.

Outbreaks of Highly Pathogenic Avian Influenza (HPAI) have been reported globally, causing severe disruption to poultry production. Moreover, HPAI has zoonotic potential and cases of HPAI jumping the species barrier have been reported.

Outbreaks of HPAI have severely impacted global foie gras production with a reduction from 28,000 tonnes in 2018 to approximately 20,000 tonnes in 2024.

In Australia and New Zealand, avian influenza (AI) is a notifiable disease. Both Australia and New Zealand were AI free, however, New Zealand reported a first outbreak of HPAI in 2024.

Gourmey´s cell cultured duck is produced using a highly controlled and hygienic biomanufacturing technology, using cells that have been rigorously tested to show they are free-from viruses such as avian influenza and the finished cell-cultured duck product is tested to show absence of food-borne pathogens such as *Campylobacter* spp., *Salmonella* spp., and *Listeria monocytogenes,* thus reducing the risk to public health and the domestic poultry population compared to conventionally produced poultry.

In 2022, CSIRO published a protein roadmap which was written in collaboration with government and industry, which identified a $13 billion market opportunity for Australia to grow and diversify its high-quality protein products from various different sources, including cell cultured meat. In this context, the Australian Prime Minister, Anthony Albanese, also called for more advanced manufacturing capabilities in Australia.

Gourmey aims to support protein diversification by offering consumers in Australia and New Zealand high quality, safe and nutritious foods derived from cell culture.

At the time of submission, Gourmey has also sent applications to the US Food & Drug Administration (FDA), Singapore Food Agency (SFA), the Federal Food Safety and Veterinary Office in Switzerland (FSVO), the UK Food Standards Agency (FSA) and the European Commission (EC) and European Food Safety Authority (EFSA). All applications are currently under review by the respective authorities.

4.1. Regulatory impact information

4.1.1. Costs and benefits of the application

a) Considerations for the consumer

Gourmey's cell cultured duck is not intended to fully replace conventional meat but to provide a safe, alternative choice to conventional duck products. It is expected that customers will diversify their protein consumption to include cell cultured duck which may displace the consumption of conventional duck meat products slightly. Hence, there will likely be no or negligible impact on the price of conventional meat products.

As demonstrated in Section E, cell cultured duck has an adequate amino acid profile and consists of nutritionally safe amounts of minerals and vitamins when compared to conventional duck. Hence, it can safely be consumed as part of a balanced and varied diet.

Rigorous controls are implemented in the preparation of Gourmey's cell cultured duck, including the nature of the cell bank sourcing and supplier controls, a robust cell bank acceptance criteria process, the production process and materials used which greatly reduces the risk profile and has advantages over the production of conventional meat which is subject to risks such as the incidence of food-borne pathogens such as *E. coli*, and *Campylobacter*.

Moreover, Gourmey´s flagship product, cell cultured "foie gras" will offer consumers access to a product that is not widely available in Australia and New Zealand. More information on consumer understanding and considerations is given in Section F of this dossier.

b) Considerations for industry

Regulatory approval will reassure consumers about the safety and quality of cell cultured meat which may lead to the potential increased customer demand, creating more opportunities for those working in the industry. The approval of cell cultured animal products can also promote sustainable food production practices.

Gourmey is planning to place their flagship cell cultured duck "foie gras" product on the market by directly distributing the product to restaurants allowing them to increase their range of options available and target a wider customer base, particularly consumers with dietary restrictions.

[redacted]

[redacted]

c) Considerations for the government

Supporting the development of cell cultured meat products can also help the government to enhance its global position leading their country towards more sustainable and innovative food systems and meet government goals for protein diversification.

4.1.2. International trade

Food Standards Australia New Zealand (FSANZ) has carried out their initial assessment for Cultured Quail as a Novel Food submitted by Vow (application A1269) and concluded that the ingredient is safe to consume. The regulatory approval of Gourmey's cell cultured duck will contribute to Australia/New Zealand's position as a leader in the growing cultivated cell cultured food industry positively affecting trade by allowing these countries to trade without barriers with other jurisdictions where these products have been approved. As indicated above, Gourmey has submitted applications for obtain regulatory approval in other jurisdictions and hence upon successful approval, this will create trading opportunities for market expansion through the import and export of Gourmey's cell cultured duck.

Moreover, as stated above, conventional foie gras production has been banned in Australia, and currently, only processed and shelf-stable forms are permitted for import as there are no biosecurity measures that apply to raw poultry. Due to the controlled and hygienic production process, Gourmey´s cell cultured "foie gras" will reduce the risks associated with the importation of raw poultry products.

5. Information to support the application

5.1. Data requirements

This application is prepared in accordance with the relevant sections of the FSANZ Application Handbook (FSANZ, 2024a) which include:

- Chapter 3.1 – General requirements for applications
- Chapter 3.5 – Guidelines for applications for new foods
    - Chapter 3.5.2 – Novel Foods

The current application contains the necessary information and data relevant to support the safety of the novel food enabling the objectives addressed in Section 18 of the FSANZ Act to be addressed accordingly.

Literature search strategy

Much of the information included in this application to support the safety of the ingredient under evaluation has been retrieved by carrying out several extensive literature reviews addressing the toxicological profile of media ingredients and duck proteins. The literature searches were conducted according to E1 Data requirements outlined in the FSANZ Application Handbook and in accordance with the EFSA guidance on systematic literature reviews (EFSA, 2010). The following literature searches were performed:

- Extensive systematic literature review on the toxicological profile of 2-mercaptoethanol through the oral route
- Extensive systematic literature review on the toxicological profile of dimethyl sulfoxide through the oral route
- Extensive systematic literature review on the toxicological profile of ethanolamine through the oral route
- Extensive systematic literature review on the toxicological profile of para-aminobenzoic acid through the oral route
- Systematic review protocol– safety review of medium ingredients (pluronic acid and insulin)
- Extensive systematic literature review on the toxicological profile of duck proteins

A detailed description of the strategy for each search has been provided along with the results of each search in Appendix 21. A list of the references used throughout this application is provided at the end of this document.

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

6. Assessment procedure

Gourmey considers the Major Procedure (Subdivision F of the FSANZ Act) to be the most appropriate assessment procedure for this application. This is due to the history of consumption of duck meat in Australia and New Zealand and the comparability of Gourmey's cell cultured duck with conventional duck meat. However, Gourmey recognises the additional scientific complexity that may be required in assessing cell cultured foods due to the novel method of food production.

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.

Gourmey has submitted a confidential and non-confidential version of the dossier.

The information contained within the following appendices is not publicly available and Gourmey requests that the information contained within these appendices is treated as confidential commercial information (CCI).

- Appendix 3 Food Safety Plan [CCI]

The food safety plan reveals sensitive details of the production process and its control. The public disclosure of the information for which confidentiality status is requested will harm the interests of Gourmey to a significant degree. A non-confidential description on the food safety management is provided in Section B.4.

- Appendix 4 Information on the Source and History of the Cell line [CCI]
- Appendix 5a Media Composition & Risk Assessment [CCI]
- Appendix 5b Extensive Media Component Risk Assessment [CCI]
- Appendix 5c i CoAs early isolation [CCI]
- Appendix 5c ii CoAs FSCM [CCI]
- Appendix 6a Master Cell Bank Results [CCI]
- Appendix 6b Methods and validation of Cell Line, WCB and MCB [CCI]
- Appendix 7 Working Cell Bank Results [CCI]
- Appendix 9 Growth Stability Report [CCI]
- Appendix 10a Pluripotency Assay Report Shake Flask Cultivation [CCI]
- Appendix 10b Pluripotency Assay Results Bioreactor Cultivation [CCI]
- Appendix 10c Karyotyping Results [CCI]
- Appendix 11 Pluripotency Assay Validation Report [CCI]
- Appendix 12 Embryonic Body Assay Report [CCI]
- Appendix 14 Pre-Harvest Sterility Confidential [CCI]
- Appendix 16a Recombinant Insulin COA Confidential [CCI]
- Appendix 16b Recombinant Insulin Sequence Confidential [CCI]
- Appendix 17 Absence Host DNA Insulin [CCI]
- Appendix 18 Absence Host Strain Insulin [CCI]
- Appendix 19 WGS Production strain [CCI]
- Appendix 22 Methods of analysis [CCI]
- Appendix 24 Process parameters and operational limits [CCI]
- Appendix 25 Supplier statement on Absence of Human Proteins [CCI]

The information contained within these appendices contains detailed descriptions of the production process, starting substances and preparations and how they are used to manufacture cell cultured duck which represents highly sensitive information which if disclosed will significantly harm the interests of Gourmey. The above documents also contain details of commercial links between Gourmey and its suppliers that should be

kept confidential. Gourmey has invested in the development and validation of methods and the methods used by Gourmey and their suppliers and how they are applied to the samples represent Gourmey's know-how and it is of utmost importance that our know-how, is protected.

A non-confidential description of the production process including source, cell line and cell bank and an assessment of the cell culture media is provided in Section B.4. and C.2, respectively.

- Appendix 8 Composition & Purity [CCI]
- Appendix 13 Stability of Duck Cells Refrigeration [CCI]
- Appendix 20a Transcriptomics Report [CCI]
- Appendix 20b Transcript_Abundances_Matches [CCI]
- Appendix 20c blastpmin35pc80aa [CCI]
- Appendix 20d diffExpr.P0.001 C2.matrix [CCI]
- Appendix 20e HEATMAPS [CCI]
- Appendix 20f biomass up seqs-megablast-ref-annotated [CCI]
- Appendix 20g Trinotate report [CCI]
- Appendix 20h biomass up seqs-megablast-rna [CCI]
- Appendix 23a Transcriptomics Toxicity Assessment [CCI]
- Appendix 23b FR003143 suppl1 blastp swissprot toxin [CCI]
- Appendix 23c FR003143 suppl2 blastp trembl toxin [CCI]

Appendices 8, 13, 20a-h and 23a-c are requested to be kept as confidential as they contain highly detailed and sensitive information about the nature and the composition of the novel food along with details on the methods and commercial links which is requested to be kept confidential.

A non-confidential summary of the composition, purity and stability of cell cultured duck is given in Section B.5 and B.6. A non-confidential summary of the transcriptomics testing is given in Section C.5.

8. Other confidential information

Gourmey requests confidential treatment for:

Appendix 15 External Laboratories Certifications

This document includes details of the commercial relationships between Gourmey, and the laboratories involved in analysing the novel food. This information is not publicly available and known only to a limited number of people and is considered to be a trade secret.

While the findings of Appendix 21 Literature Reviews are public information, the document includes personal details that the applicant wishes to keep confidential. Therefore, a non-confidential version with the personal details redacted has been provided along with this application.

No other confidential information is included in this application.

9. Exclusive capturable commercial benefit (ECCB)

Gourmey´s cell cultured duck is produced using a proprietary production process hence, 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

At the time of submission, there are no Codex Alimentarius Commission standards that are relevant to Gourmey's cell cultured duck.

10.2. Other national standards or regulations

At the time of submission there are no other national standards or regulations related to Gourmey's cell cultured duck.

11. Statutory declaration

A signed Statutory Declaration is provided in Appendix 1.

12. Checklist

A complete checklist is provided in Appendix 2.

NOVEL FOODS

A. Exclusive use of novel foods

Suprême SAS (Gourmey) is not seeking exclusive permission for the use of cell cultured duck should it be approved by FSANZ as a novel food. Gourmey´s cell cultured duck is a new product to the market and has been produced using a production process with no previous history of use in food. It will be commercialised under a trade name that has not yet been decided. The legal descriptor we propose is 'cell cultured duck'.

B. Technical information on the novel food

Technical information on Gourmey's cell cultured duck is provided in this section. 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 (FSANZ, 2024a). The cell cultured duck presented in this dossier is representative of the commercial product for which approval is sought.

B.1. Information on the type of novel food

Gourmey utilizes an innovative production process to produce cell cultured duck, which is intended to be used as a food ingredient when combined with other suitable food ingredients to be mixed with other authorised ingredients to be used in duck meat analogues such as "cell cultured foie gras" and "cell cultured duck pâté". The scope of this dossier does not include the final or finished products. Gourmey's cell cultured duck is produced using a semi-continuous production process, developed specifically for the safe and consistent production of cell cultured duck products.

The cell cultured duck is obtained from duck embryonic stem cells (dESC) isolated from fertilised duck eggs from the species *Anas platyrhynchos domesticus* (Pekin duck) obtained from a commercial duck breeding flock reared under standard farming conditions and in accordance with animal health and welfare legislation applicable in the European Union. The novel food is a pale pink/beige biomass and contains a minimum of 10% protein (70% protein on a dry basis). The production process consists of creating a master cell bank and working cell banks under sterile conditions using non genetically modified cells from fertilised eggs that are stable and free from microbial contamination and adventitious agents. The biomass is produced under sterile cell culture conditions in which the cells are grown in a chemically defined food safe medium that does not contain antibiotics or foetal bovine serum. The harvested cells are washed in saline solution and then frozen until further use.

Gourmey has fully characterized the cell line used to create the Master Cell Bank (MCB) and the Working Cell Bank (WCB), which represent the starting point of cell cultured duck production. Analytical data confirm the identity, integrity, stability and sterility of the cell line and production cell banks along with the acceptance criteria for their release. Good Cell Culture Practice 2.0 (Pamies et al., 2022a) 21 CFR § 117 subpart B, § 192 and § 592 and ICH Q5D (ICH, 1997), relevant principles of 21 CFR § 210, the FSANZ Standard 3.2.2 and EU Regulations (Regulation (EC) No 852/2004 and Regulation (EC) No 853/2004) were followed in the context of food production and the principles of Good Manufacturing Practice (GMP) have also been considered and followed to ensure appropriate hygienic conditions in the applicable laboratories and workspaces.

Taxonomic information on the source species of the duck eggs can be found below:

Class: Aves
Order: Anseriformes
Family: Anatidae
Genus: *Anas*
Species: *A. platyrhynchos*
Subspecies: *A. p. domesticus*
Breed: Pekin duck

Gourmey's cell cultured duck fall within the following major 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

The ingredient cell cultured duck is intended for use as a human food ingredient when combined with other suitable food ingredients to be used in meat imitates, spreadable-textured specialties, fats, oils and derivatives, and duck meat analogues such as "cell cultured foie gras" and "cell cultured duck pâté". The subject of this dossier is cell cultured duck as a food ingredient. Details of finished products are not presented, although the use of cell cultured duck in different food categories is contemplated in the intake assessment (Section D.3). Gourmey's cell cultured duck is produced using a semi-continuous production process, developed specifically for the safe and consistent production of cell cultured duck products.

The cell cultured duck will be used as a food ingredient in the manufacture of cultured pan-seared foie gras and other cell cultured duck meat analogues. The proposed inclusion rate of cell cultured duck is between 5 – 80% by weight depending on the finished product.

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

Gourmey's cell cultured duck is derived from duck embryonic stem cells (dESC) isolated from fertilised duck eggs from the species *Anas platyrhynchos domesticus.* The ingredient is not a chemically defined molecule or a mixture of known chemical elements. Full details on the source and composition are described in Sections B.4.2 (Cell Line Establishment and Creation of the Cell Banks), B.5 (Information on the impurities) and B.6.2 (Batch analysis).

B.4. Manufacturing process for a novel food ingredient

B.4.1. Introduction to the manufacturing process and flowchart

A systematic analysis has been performed at each stage of the production process to identify and characterise potential food safety risks that could be inherent in the cells themselves or introduced during the production process. All stages of the production process have been described along with the approach taken to determine safety. This dossier describes the basis for determining that the cell line, biomass production (including all culture media components and other inputs utilized in the process) and the final cell cultured duck as an ingredient is safe for human consumption.

A confidential version of the production process together with the Hazard Analysis and Critical Control Point (HACCP) plan is presented in Confidential Appendix 3 - Food Safety Plan.

An overview of Gourmey's production process can be found below in Figure 1.

The entire production process is comprised of four broad phases:

- The creation of the Master Cell Bank (MCB) and Working Cell Bank (WCB).
- Biomass production.
- Cell harvest, washing, and packaging.
- Final product formulation (this step is not covered in the dossier).

The process starts with the reception of a qualified cell bank (for information on the process and passage number, please refer to Confidential Appendix 4 - Information on the Source and History of the Cell line on the production of the MCB following the principles of GMP in Regulation 852/2004 and Regulation 853/2004, Good Cell Culture Practice 2.0 (Pamies et al., 2022b) and ICH Q5A, ICH Q5C and ICH Q5D where applicable to food production.

Rigorous testing is performed on the MCB to validate the identity, sterility, and stability of the cell line. Once the MCB is validated, a WCB is created as a practical starting point for the production process of cell cultured duck.

The production process is defined as semi-continuous as multiple independent harvests are possible from a single WCB vial due to a process referred to interchangeably by Gourmey as continuous seed train or parallel passaging. In this process, the cells from a WCB vial are thawed and placed into shake flasks with culture medium. A small volume of the pooled cells is retained from the shake flasks and kept in culture in parallel to the next phases. The culture medium is manufactured independent of the semi-continuous process and is stored until it is ready for use in production. During the production process, the cells are continuously replenished with fresh media from the same batch of prepared media as required until the media batch is used up. One batch of medium can be used for multiple separate harvests with continuous passaging of the cells.

These shake flasks can be used as a starting point for subsequent independent harvests. This semi-continuous process optimizes the production process as a new WCB vial does not need to be thawed for each harvest. The stability of the cells during this semi-continuous process has been evaluated and data to support the stability of this process are presented in Section B.6.3.

Cells from the shake flask seed train are used to inoculate the N-1 Suspension Bioreactor (SBN-1), where they are grown under controlled conditions until the desired cell density is reached after which, the majority of the culture volume is used to inoculate an N Suspension Bioreactor (SBN).

A small volume of the cells is retained and used to maintain a continuous seed train in the SBN-1 bioreactor once it is replenished with culture media in addition to the shake flask seed train. This continuous seed train in the SBN-1, is a process optimization that allows to seed and harvest the SBN bioreactor more frequently and reduces the frequency needed to start the process from a WCB vial.

Once the culture in the SBN reaches a desired cell density, the biomass is harvested from that bioreactor. Once the bioreactor has been harvested, it can be re-seeded from the step prior as a continuous seed train would have been maintained.

The last phase of production relates to the harvesting of the cell cultured duck. The biomass is separated from the culture media by centrifugation and washed with saline solution under centrifugal force within the same device. The biomass is transferred to food-grade bags, vacuum packed and frozen.

The cells are kept frozen, until they are used to produce a final product, where the cells will be combined with other food-safe ingredients. The manufacture of finished products containing cell cultured duck, is not covered in the scope of this application.

All steps of the production process are conducted following appropriate hygienic techniques and in line with food safety principles. The Food Safety Plan (FSP) contains all key information about the hazard identification, risk analysis and preventive and corrective measures applied to each hazard identified. More details regarding Gourmey's FSP along with a detailed flowchart illustrating the production process are provided in the Confidential Appendix 3 - Food Safety Plan.

Gourmey is currently producing their product at a scale of 200L. The maximum volume that Gourmey intends to produce at will be 2,500L, which is ca. 10 x the volume of the current bioreactor that was used to generate the batches that support this dossier. In the scenario where a 2,500L bioreactor is used for production, the 10L becomes the suspension bioreactor (SB) N-2, the 200L becomes SBN-1, and the 2,500L becomes the SBN (the largest volume bioreactor). The process activities described in this section of the dossier comprise the same steps that would be performed to inoculate from a 200L bioreactor to a 2,500L bioreactor. As such, the increase in scale is not expected to introduce new food safety concerns because the transfer steps described are the same activities for 200L to 2,500L. The harvest, washing, and packing activities described would also be the same with the only key difference being scale. As the activities are the same, the expected hazards would have already been addressed. It is acknowledged that the scale-up may affect the risk assessment for the previously identified hazards. Therefore, as Gourmey scales up, they will carry out a thorough review of the Food Safety Plan to ensure that the cell cultivated duck meets the same current specifications with regards to identity, stability, composition, and impurities. The highly controlled conditions currently used by Gourmey including the maintenance of sterility and rigorous monitoring of the growth environment will remain the same. Gourmey's internal SOPs ensure that the same safety standards will be upheld at larger scales, reducing variability. Consequently, no additional hazards beyond those outlined in the Food Safety Plan, for which risk mitigation measures have been implemented, are expected.

In the sections below, we provide further details on each stage of the production process, data to support the identity, stability and sterility of the cell line, and the nutritional composition and impurity testing of the cell cultured duck.

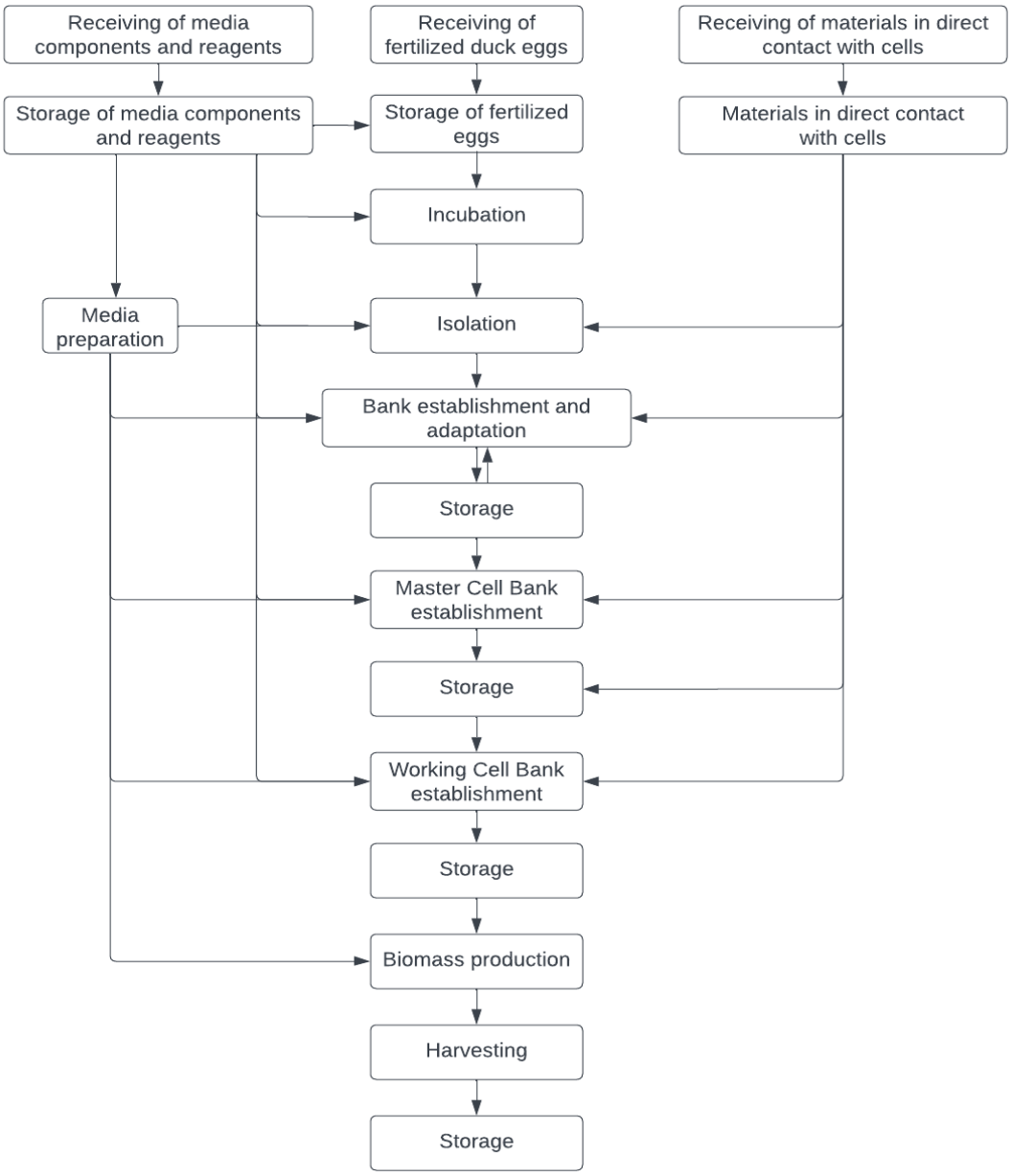

Figure 1. Overview of Production Process

B.4.2. Cell Line Establishment and Creation of the Cell Banks

Gourmey has developed a robust scientific framework to support the identity, sterility, and stability of the specific duck cell line discussed in this dossier. This involves providing details on the origin of the cell line, the components used during isolation, adaptation to suspension and cell bank establishment phases. Our strategy for demonstrating the identity, stability, and sterility of the cell line, as well as the criteria for creating and releasing our MCB and WCBs is also detailed. The criteria include ensuring consistent cell viability and doubling time, ensuring sterility (absence of mycoplasma, mould, yeast, bacteria, and adventitious viruses) and ensuring the identity of and stability of the cell line.

B.4.2.1. Cell Origin

The cell line used to produce Gourmey's cell cultured duck was isolated from fertilized duck eggs from the species *Anas platyrhynchos domesticus* (Pekin duck) obtained from a commercial duck breeding farm based

in the EU (with no history of avian influenza (AI)) and reared under standard farming conditions and in accordance with animal health and welfare legislation applicable in the European Union.

It is important to note that the eggs were obtained before the most recent outbreaks of AI that we have seen in Europe. The farm rears ducks and chickens for breeding/laying and supplies farms with eggs which are hatched, and the birds are subsequently reared for duck meat. The flock was certified as free from avian flu and *Salmonella* spp., and no *in ovo* vaccinations were performed. The laying birds received the basic vaccination package in the EU:

- *Salmonella enterica*;
- *Salmonella typhimurium*;
- *Riemerella anatipestifer;*
- Duck viral enteritis (*Anatid alphaherpesvirus* 1 or AnHV-1)
- Duck hepatitis (*Avihepatovirus*)

The batch of eggs was accompanied by a declaration from the manufacturer, the full vaccination schedule record of the laying ducks can be found in Confidential Appendix 4 - Information on the Source and History of the Cell line. The surface of the eggs was carefully cleaned and disinfected with a mild detergent before being placed in an incubator.

During cell isolation, laboratory staff wore protective equipment consisting of single-use lab coats, masks, gloves, hair nets, and shoe covers to limit the risk of cross-contamination. Antibiotics (streptomycin and penicillin) were used in the primary cells to guarantee sterility and protect them from bacteria and fungi.

All activities were conducted in biosafety cabinets to minimise the risk of contamination.

Taxonomic information on the source species of the eggs can be found below:

Class: Aves
Order: Anseriformes
Family: Anatidae
Genus: *Anas*
Species: *A. platyrhynchos*
Subspecies: *A. p. domesticus*
Breed: Pekin duck

Gourmey's cell cultured duck is derived from dESC isolated from fertilized eggs belonging to the species described above. The cells used in Gourmey´s production process are pluripotent ESCs, meaning that they can indefinitely self-renew (proliferate while preserving their undifferentiated nature) (Farzaneh et al., 2017). The duck cells are not differentiated during the production process.

ESCs by their nature exhibit many intrinsic properties that enable them to proliferate extensively in culture compared to somatic cells such as fibroblasts. Gourmey's cell line has not been genetically modified or edited to induce immortalisation, nor has it been subjected to any protocol to select for spontaneous immortalization. The inherent properties of ESCs include sustained pluripotency and a sustained proliferation. The short cell cycle of ESCs facilitates rapid proliferation, maintaining the cells' undifferentiated state across numerous passages. The inherent pluripotency of ESCs, coupled with their high telomerase activity, ensures that they can replicate without succumbing to the telomere shortening that typically limits cellular lifespan (Hayflick limit). This characteristic, combined with their genetic stability, contributes to the longevity of ESC cultures, enabling them to undergo many more passages than cells such as fibroblasts. Although subject to natural mutations with every cell division and with that adapted to suspension media, the cells used are not immortalised as they still can differentiate. Without the addition of exogenous growth factors that would trigger differentiation, ESCs exist in their undifferentiated state, and are therefore stable in the cell culture medium.

B.4.2.2. Cell line identity, stability and sterility

B.4.2.2.1. Cell line identity

To confirm species identity, quantitative polymerase chain reaction (qPCR) was performed on the cells following validated methods in an accredited laboratory. The cell line was also tested to confirm absence of cross-contamination with 13 other species (e.g., murine, porcine, bovine and chicken) using the same qPCR assay. The results confirmed that the cell line belongs to the species *A. platyrhynchos* and has not been cross contaminated by other species. Species identification was performed on the cells from the qualified bank, MCB and WCB (Confidential Appendices 4, 6 and 7).

Additionally, karyotyping was performed on cells from the MCB, WCB and End of Production (EOP) and the results confirmed that the cells were from *A. platyrhynchos* and showed normal ploidy (Confidential Appendix - 10c Karyotyping Results).

B.4.2.2.2. Cell line stability

Avian embryonic stem cells (ESC) are diploid and can be cultured continuously when the culture conditions are stable and can sustain the proliferation of the cells.

Cell cultures are under stress due to the artificial environment in which they are placed and the techniques they are exposed to; hence, there is a risk of genomic instability in cell lines due to constant sub-culturing (or passaging), where mutations can build up over time that may eventually cause changes in the phenotype of the cells (Soice and Johnston, 2021, Li et al., 2019). The use of quality-controlled cell banks of cryopreserved cell lines is a way to mitigate the risk of losing cell-line fidelity caused by genetic drift, as well as protecting against the presence of viruses, bacteria, moulds, yeast and *Mycoplasma* spp. (FAO, 2023). Furthermore, monitoring the phenotypic characteristics of the cells can identify deviations that may impact the integrity of the cells and the production process. Therefore, the primary phenotypic and genotypic characteristics of the cell line were determined, and an appropriate panel of tests were used to ensure their consistency and stability. The primary characteristics of the cells and the panel of tests are discussed further below. A summary of the different tests used to confirm stability are presented in Table 1.

B.4.2.2.2.1. Phenotypic characteristics

The main characteristic of ESCs is pluripotency. Several signalling pathways operate in ESCs to regulate the balance between self-renewal and differentiation. A network of transcription factors (TFs) is involved in the establishment and maintenance of pluripotency including POU5F3 (also known as OCT4), DNMT-3B and NANOG, which regulate pluripotency by integrating positive and negative signals to repress differentiation and sustain self-renewal of pluripotent stem cells (Pain et al., 2018). Additionally, telomerase reverse transcriptase (TERT) expression plays a crucial role regulating ESC self-renewal and pluripotency (Lupatov and Yarygin, 2022).

The pluripotent state of ESCs can be assessed by examining the transcriptomic expression of recognised genetic markers documented in literature. Investigating gene expression is relevant as the transcriptome governs the cell's proteome and consequently, its phenotype.

A further characteristic of pluripotent cells is their ability to form embryoid bodies (EB) when exposed to specific conditions (Lin and Chen, 2014). EB are self-organizing three-dimensional aggregates formed in suspension by pluripotent stem cells (PSCs) (Kurosawa, 2007). The process of EB generation and differentiation mimics some aspects of early embryonic development and gives rise to structures that contain derivatives of the three germ layers. Although most research has been carried out with human and mouse PSCs, the same concept and methodology can be applied to any other species. Examples in birds are rare, but EB differentiation has been performed with chicken (Wu et al., 2010) and duck PSCs (Guan et al., 2010). Consequently, EB differentiation can be applied as a functional *in vitro* assay to assess the trilineage potential of dESCs, thus validating their pluripotency.

The expression of the transcription factors described above and the ability of the cells to form EB have been selected to confirm the phenotypic characteristics of the cell line and to demonstrate its stability.

The cell line was tested at different stages of the production process for its capacity to express pluripotency markers as well as to lose expression of pluripotency markers in favour of the expression of early development markers of the three germ layers. Further details regarding the stability of the cells during the production process are provided in Section B.4.2.2.2.3.

The following growth characteristics were also used to confirm the phenotypic characteristics of the cells:

- Cell viability: It is important to monitor this parameter as the process yield and success depends on the percentage of live cells. Abnormal values may also indicate some cellular stress to process conditions, defective media ingredients or contamination.
- Viable Cell Density (VCD): Is an important parameter used to quantify the density of viable cells in the culture. The VCD indicates the viability of the cells and a reduction in VCD can indicate cellular stress, inadequate nutrients or contamination.
- Doubling Time (DT): This parameter describes the proliferative capacity of the cells. An increase in DT can indicate cellular stress, inadequate nutrients or contamination.
- Cell phenotype: visual inspections under the microscope can reveal unusual morphological changes and/ lytic activity

B.4.2.2.2.2. Genotypic characteristics

Karyotyping can be useful to identify spontaneous genomic changes, including those affecting safety, as indicated by the Food and Agriculture Organisation (2023). Karyotyping consists of closely examining the chromosomes, counting their numbers and checking for abnormalities, structural alterations or changes. Most avian species like duck are diploid and contain a typical avian karyotype composed of two sets of chromosomes including macro- and microchromosomes. The Pekin duck karyotype has 80 chromosomes (9 pairs of macrochromosomes and 31 pairs of microchromosomes) (Li et al., 2021). It is important to note that karyotyping in avian cells is not as advanced as in mammalian cells and only macrochromosomes are generally examined. The size and organisational complexity of microchromosomes critically limit the data that can be obtained from their examination (Kretschmer et al., 2018). Karyotyping was performed on cells taken from the MCB, WCB and at the end of production. For each sample, 50 metaphases per sample were examined.

The panel of tests used to support the stability of the cell line are presented in Table 1. This panel of tests was selected to monitor the stability of the cell line and to ensure it maintains the characteristics of ESCs confirming that no undesired changes in pluripotency occurred during routine passaging, the creation of the cell banks and during the production process. The RT-qPCR tests developed in-house have been validated internally for the intended purposes (Confidential Appendix 11- Pluripotency Assay Validation Report).

Further details regarding the methods, frequency of testing and results are provided in Section B.4.2.2.2.3.

Table 1. Summary of Tests Performed to Confirm the Genetic Stability of the Cell Line

Type	Test	Rationale	Internal Standard Operating Procedure (SOP)
Growth stability	Viable cell density (VCD)	To quantify the density of viable cells in the culture.	SOP-SCUP-003
	Viability	To evaluate the ratio of viable cells compared to the number of dead cells (state of the culture).	
	Doubling time	The doubling time is linked to the VCD, and it is a control to assess the cell proliferation quality.	

Table 1. Summary of Tests Performed to Confirm the Genetic Stability of the Cell Line			
Type	**Test**	**Rationale**	**Internal Standard Operating Procedure (SOP)**
Phenotypic stability	Phenotype by microscopy	To assess if the cells change their morphology throughout the production run (e.g., aggregates, single cells, differentiated cells).	SOP-SCUP-007
	[redacted]	ESC transcription factor. Plays a critical role in pluripotency, self-renewal, and proliferation.	SOP-MOLB-010
	[redacted]	ESC transcription factor. Plays a critical role in pluripotency.	SOP-MOLB-010
	[redacted]	[redacted]	SOP-MOLB-010
	[redacted]	[redacted]	SOP-MOLB-010
	Embryoid body formation	The formation of an EB indicates that the cells are pluripotent	SOP-QCTL-012
Genetic stability	Karyotyping	Cytogenetic analysis to determine the ploidy of the cells	External laboratory
Notes: [redacted]			

The stability of the cell line from MCB to end of production is discussed in the following sections where data are presented to show that the cells are stable during the production process.

B.4.2.2.2.3. Stability of the cells during production

The stability of a cell line is crucial for the consistent production of cultivated meat. As outlined in Section B.4.1., the production process of cell cultured duck is defined as semi-continuous whereby multiple independent harvests are possible from a single WCB vial due to a process referred to interchangeably by Gourmey as continuous seed train or parallel passaging. The purpose of the studies presented below was to demonstrate the stability of the cell line when used in semi-continuous production.

The stability of the cell line was assessed using the different methods presented in Table 1 all of which were selected to demonstrate consistency in phenotypic and genotypic characteristics for a period of time that was representative of the semi-continuous production process described in Section B.4.3. An overview of the tests performed at each stage of production is presented in Table 2 along with the reference to each specific Appendix which describe the study design and results. Taken together, the results obtained from these analyses show that cells taken from the WCB are stable during production. From vial thaw to the end of production, the cells maintained pluripotency, normal growth characteristics and the ability to form EB which supports the stability of the cells in the semi-continuous process.

Table 2. Stability Study Plan Overview							
Production stage	**Study samples**	**Cell viability**	**Doubling time**	**Karyotyping**	**RT-qPCR pluripotency**	**EB Assay**	**Species specific PCR**
Cell bank	MCB bank	Not performed	Not performed	X Conf. Appendix 10c	X Conf. Appendix 6a	Not performed	X Conf. Appendix 6a
	MCB recovery	X Conf. Appendix 6a	X Conf. Appendix 6a	-	X Conf. Appendix 6a	-	-
Continuous shake flask culture	WCB bank	X	X	X Conf. Appendix 10c	X Conf. Appendix 7	NA	X
	Recovered cell culture from the WCB	X Conf. Appendix 9	X Conf. Appendix 9	-	X Conf. Appendix 10a	X Conf. Appendix 12	-
	Every passage	X Conf. Appendix 9	X Conf. Appendix 9	-	-	-	-
	Intermediate timepoint	X Conf. Appendix 9	X Conf. Appendix 9	-	X	X	-
	EOP	X Conf. Appendix 9	X Conf. Appendix 9	X Conf. Appendix 10c	X Conf. Appendix 10a	X Conf. Appendix 12	X
SBN-1 cultivation	Pooled shake flask culture to inoculate SBN-1	X Conf. Appendix 9	X Conf. Appendix 9	-	X Conf. Appendix 10b	X Conf. Appendix 12	-
	SBN-1 cultures that seed the SBN	X Conf. Appendix 9	X Conf. Appendix 9	-	-	-	-
	SBN-1 (day 15, ±10 days)	X Conf. Appendix 9	X Conf. Appendix 9	-	X Conf. Appendix 10b	X Conf. Appendix 12	-
	EOP SB10	X Conf. Appendix 9	X Conf. Appendix 9	X Conf. Appendix 10c	X Conf. Appendix 10b	X Conf. Appendix 12	-
SBN cultivation	Pre-harvest batch EOB RB-001B	-	-	-	-	-	-
	Pre-harvest batch EOB RB-001C	X Conf. Appendix 9	X Conf. Appendix 9	-	-	-	-
	Pre-harvest batch EOB RB -001D	X Conf. Appendix 9	X Conf. Appendix 9	-	-	-	-
	Pre-harvest EOP batch RB-001G	X Conf. Appendix 9	X Conf. Appendix 9	-	X Conf. Appendix 10b	X Conf. Appendix 12	-

[1]EB assay was not performed as it is not technically viable at this stage. To perform the EB experiment, cells must be in growth phase.

Table 2. Stability Study Plan Overview							
Production stage	**Study samples**	**Cell viability**	**Doubling time**	**Karyotyping**	**RT-qPCR pluripotency**	**EB Assay**	**Species specific PCR**
EOP = end of production; EB = embryoid body; MCB = master cell bank; PCR = polymerase chain reaction; RT-qPCR = Quantitative Reverse Transcription Polymerase Chain Reaction; SB = suspension bioreactor; WCB = working cell bank							

B.4.2.2.2.4. Growth stability

The growth stability of the cells was assessed over a period that was representative of the semi-continuous process.

Key parameters measured were the doubling time (DT), viable cell density (VCD), cell viability (Viability expressed in %), cumulative population doubling level (PDL) in shake flask culture, and cell morphology (assessed by microscopy). The investigation was conducted across the different scales (shake flasks, SBN-1 and SBN). The data were analysed to assess the consistency of growth parameters across the different stages of production.

Growth parameters were consistent (DT, viability, linear cumulative population doubling level) at each scale and over the course of a representative production run (Confidential Appendix 9 - Growth Stability Report), which supports consistent growth stability.

B.4.2.2.2.5. Pluripotency markers

The results from the MCB are presented in (Confidential Appendix 6a), results from the WCB and continuous shake flask cultivation are presented in (Confidential Appendix 10a) and results from cultivation in the bioreactors is presented in (Confidential Appendix 10b). The data show that the cells maintain the ability to express pluripotency-related gene markers throughout the course of a semi-continuous culture that is representative of Gourmey's production process. Moreover, the cells showed the same expression profile over the course of the study and at the transcriptomic level of expression, the pluripotency associated genes were highly expressed compared to the non-pluripotent negative control cell line. The internal validation of this method can be found in (Confidential Appendix 11).

B.4.2.2.2.6. Embryoid body formation

In order to facilitate the differentiation of the cells into EBs, the culture conditions were adjusted, and a specific media was used for that purpose. Gourmey assessed the capacity of the cells to form EBs by applying specific culture conditions that favour cell aggregation and formation of EBs.

The full report can be found in (Confidential Appendix 12 - Embryonic Body Assay Report), which shows that the cells maintain the ability to form EBs regardless of the stage of production, indicating that the cells are stable and maintain the characteristics of ESCs. Moreover, the cells showed the same gene expression profile over the period of the EB formation protocol. At the transcriptomic level of expression, the expression of pluripotency-associated genes is progressively lost when compared to the day -1 (corresponding to the sampling date) whilst the expression of early development-associated genes increased.

B.4.2.2.2.7. Karyotyping

Karyotyping results (Confidential Appendix 10c), MCB, WCB and EOP samples show that, for all, the modal number of chromosomes was 78-79 confirming and don't present any ploidy abnormalities. This confirms the identity of the cells as duck cells and that the genome of cells is stable.

B.4.2.2.3. Cell line sterility

Cell sourcing, isolation, passaging and storage may introduce microbial contamination that could be propagated during subsequent phases of production. A potential hazard is the transmission of zoonotic

infectious diseases and foodborne pathogens from the source of the cell line, which in this case is fertilized duck eggs, although the chances are considerably lower compared with conventional livestock breeding (Treich, 2021).

Cells are at risk of microbial contamination at each stage of production; hence all activities are performed under appropriate hygienic conditions. Among the bacteria that commonly infect eukaryotic cell lines, *Mycoplasma* spp. is a major concern, as several species are known human pathogens and can cause cell cultures to fail and are difficult to eradicate during biomanufacturing (FAO, 2023, Farzaneh et al., 2017). During production, contamination from other bacteria, yeast and fungi from the production environment can also occur, particular attention and control must be directed to spore-forming bacteria and fungi that are challenging to eliminate and can be persistently transmitted in the air (Møretrø and Langsrud, 2017, Snyder and Worobo, 2018, FAO, 2023). The risk of contamination by viruses and infectious prions also exists when animal-derived components are used (Hadi and Brightwell, 2021, Ong et al., 2021). To limit the occurrence of contamination, early detection via regular monitoring is critical, control and supplier verification activities as well as following good hygiene practices throughout the entire production process.

The cell line has been tested at different stages to confirm the absence of microbial contamination and adventitious agents. The risk of contamination has been controlled by:

- The laying flock from which the cell line was derived was routinely vaccinated. The ducklings were vaccinated against *E. coli*, *Pasteurella multocida* and duck viral hepatitis. The adult birds were vaccinated against *Reimerella anatipestifer,* Avian metapneumovirus, duck viral hepatitis and *Salmonella enteritidis*.
- The flock and environment were regularly tested (swabs and faecal samples) for the presence of *Salmonella* spp. in accordance with EU farming practice.
- Upon receipt of the eggs, the batch was visually inspected and thoroughly washed in a mild disinfectant to remove faecal matter and any residual pathogens present on the eggshells.
- A homogenised sample of the yolk and egg albumen from the batch of fertilized eggs used to isolate the cell line was sent for microbiological analysis (*Salmonella* spp., *E. coli, Proteus, Pseudomonas* spp.*, Staphylococcus* spp.). No pathogens were detected.
- The cell line was isolated under strict aseptic conditions and antibiotics were used in the early stages of isolation to protect the cells.
- Animal-derived components were kept to a minimum and only used when strictly necessary.
- The embryonic fibroblast cells used for the 2D culture were tested to ensure sterility.
- Recombinant growth factors were used to minimize the risk of transfer of animal-derived microbial contamination.
- Implementation of different programs in line with the Hazard Analysis and Critical Control Point (HACCP), Hazard Analysis and Risk-based Preventative Controls (HARPC) and current Good Manufacturing Practice (GMP) principles (e.g., supplier management, sanitation, etc.).
- Training of all laboratory staff in good laboratory and hygiene practices.
- Strict environmental monitoring of laboratory workspaces.
- Good Cell Culture Practice (GCCP) 2.0 (Pamies et al., 2022a) was followed during the cell line isolation, adaptation and banking stages.
- Rigorous testing of MCB and WCB in line with GCCP and based on the principles of ICH Q5A, ICH Q5C and ICH Q5D where applicable to food production.

Moreover, the sterility of the environment, equipment and auxiliary materials were closely monitored to ensure the operations were carried out under appropriate hygienic conditions. The cell line has been routinely tested at different stages for microbial contamination. It should be noted that most fungal or bacterial contamination would become visible within a short timeframe depending on the species, and any contaminated cell cultures would be quickly identified and adequately discarded. Due to the unique characteristics of *Mycoplasma* spp., separate tests were performed to investigate the potential presence of this microorganism. The presence of viruses was also evaluated during MCB creation.

More details can be found in Sections B.4.2.3 and B.4.2.4.

B.4.2.3. Creation and validation of the master cell bank

Cells from a qualified cell bank were adapted from R&D reagents and culture media components which were appropriate for biological processes and cell requirements during the sensitive stage of isolation, to culture media components that are acceptable and safe for use in food production (Confidential Appendix 5 - Media Composition and Risk Assessment). The adapted cells were used to create the MCB.

The MCB was manufactured following the GMP principles of Food Standard 3.2.2, 21 CFR § 117 subpart B, 21 CFR Parts § 192 and § 592, Regulation (EC) No 852/2004, and Regulation (EC) 853/2004. GCCP 2.0 (Pamies et al., 2022b) and ICH Q5A, ICH Q5C and ICH Q5D principles were also applied where applicable to food production. The storage, maintenance, characterization, and culture of cells or cell cryobanks is in line with the general principles GCCP 2.0, specifically, the MCB is maintained in a secure and orderly manner, at a temperature and by a method that will retain the initial characteristics of the cells and ensure freedom from contamination and deterioration.

The process followed for the MCB creation is described below.

In brief, cells were expanded and closely monitored until sufficient a volume, viability, and cell density were achieved. All steps of the MCB production were performed in accordance with internal protocols to guarantee the uniformity of all vials produced. The staff involved in the creation of the MCB received training on all relevant procedures including documentation control, environmental monitoring, gowning and cleaning. Each procedure followed during the production of the MCB was documented to guarantee the traceability and quality of the process. Frequent environmental monitoring was performed to confirm the microbiological quality of the environment and surfaces and to ensure that aseptic conditions were maintained during the MCB manufacturing process (Table 3).

The MCB was prepared using a proprietary chemically defined food-safe medium including bovine recombinant insulin. The composition of the food-safe medium can be found in Confidential Appendix 5 - Media Composition and Risk Assessment.

The MCB was extensively characterized and tested during and after production to ensure the absence of adventitious agents that may pose a risk to the cell line itself and the consumer. Before the final banking, vials (referred to as ‘Pre-MCB’) were analysed internally to monitor the process conditions and quality of the cells. This included rapid tests for *Mycoplasma* spp. detection, confirmation of phenotypic characteristics under the microscope, assessment of pluripotency markers and doubling time (DT). All tests performed to validate the MCB were outsourced to a Good Manufacturing Practice (GMP) accredited laboratory to guarantee the reliability and quality of the results (Table 4).

MCB vials are stored at < -150 ºC (-238 ºF) in dedicated liquid nitrogen tanks or ultra-low temperature freezers located in a controlled access area. To prevent cross-contamination with other cell lines and preserve the unique characteristics of the cells, no other cell lines are kept within the same tank. The tanks are fitted with sensors that alert the laboratory staff if the temperature rises or if the liquid nitrogen runs low. Tanks are stored in a dedicated area with restricted access only to trained staff (via a lock on the tank).

Table 3. Overview of Microbiological Sampling for Environmental Monitoring Program Followed during MCB Manufacture					
Area	**Process location**	**Type of sample**	**Method**	**Frequency**	**Specification**
Aseptic area 1 - MCB and WCB banking area	BSCs	Active viable air	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	<1 cfu/ m^3
		Viable particles from surfaces	Contact plate SOP-QCTL-006 FORM-QCTL-010	Weekly	<1 cfu/25 cm^2
		Passive viable air	Settle plates: TSA and SDA plates SOP-QCTL-002 FORM-QCTL-002	Weekly	<1 cfu/15 min
	Incubator(s)	Viable surface (shaking tray/inside door)	Contact plate SOP-QCTL-006 FORM-QCTL-010	Monthly	Negative
	Main lab area	Active viable air	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	< 200 cfu/ m^3
		Viable particle from floor	APC swab SOP-QCTL-001 FORM-QCTL-001	Weekly	At rest: < 25 cfu/25 cm^2 in operation: < 50 cfu/25 cm^2
Aseptic area 2 - Seed train and biomass production area	BSCs	Active viable air particles	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	< 1 cfu/ m^3
		Viable particles from surfaces	Contact plate SOP-QCTL-006 FORM-QCTL-010	Weekly	< 1 cfu/25 cm^2
		Passive viable air	Settle plates: TSA and SDA plates SOP-QCTL-002 FORM-QCTL-002	Weekly	< 1 cfu/15 min
	Bioreactors	Viable particles surface	Contact plate SOP-QCTL-006 FORM-QCTL-010	In operation	At rest: < 5 cfu/25 cm^2 in operation: < 25 cfu/25 cm^2
	Main lab area (inoculation, bioreactor and harvest room)	Active viable air	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	< 100 cfu/ m^3
		Viable particle floor	Contact plate SOP-QCTL-006 FORM-QCTL-010	Weekly	At rest: < 5 cfu/25 cm^2 in operation: < 25 cfu/25 cm^2

Table 3. Overview of Microbiological Sampling for Environmental Monitoring Program Followed during MCB Manufacture					
Area	**Process location**	**Type of sample**	**Method**	**Frequency**	**Specification**
	Tubing used during harvest	Surface outlet tubing	APC swab	In operation/ pre-harvest	< 1 cfu/swab
Media preparation room	BSCs	Active viable air particles	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	< 1 cfu/ m^3
		Viable particles from surfaces	Contact plate SOP-QCTL-006 FORM-QCTL-010	Weekly	< 1 cfu/25 cm^2
		Passive viable air	Settle plates: TSA and SDA plates SOP-QCTL-002 FORM-QCTL-002	Weekly	<1 cfu/15 min
	Main lab area	Active viable air, non-viable air and viable surface	Air sampler: TSA and SDA plates WI-QCTL-001 FORM-QCTL-005	Weekly	< 200 cfu/ m3
Pilot facility	4 sampling sites from the sampling list	Surface sample	Enterobacteriaceae swab	Weekly/ In-operation	< 30 cfu/swab
		Surface sample	*Listeria monocytogenes swab*	Weekly/ In-operation	Negative
		Surface sample	*Salmonella spp swab*	Weekly/ In-operation	Negative
Notes: BSC = Biosafety Cabinet; APC = Aerobic Plate Count; cfu = Colony Forming Units; TSA = Tryptic Soy Agar; SDA = Sabouraud Dextrose Agar					

Vials from the MCB were sent for analysis to validate the identity and sterility of the MCB as outlined in Table 4. The panel of tests selected were validated for their intended purpose. All external analyses were performed at GMP accredited laboratories (Confidential Appendix 15 - External Laboratories Certifications). Internal RT-qPCR tests were validated in-house (Confidential Appendix 11 - Pluripotency Assay Validation Report).

The rationale for each test is provided below:

- Cell identity: the identification at species level is necessary to ensure that the cell culture has not been contaminated with cells from another species and/or cell lines.

- Cell performance indicators:
  - Cell viability: It is important to monitor this parameter as the process yield and success depends on the percentage of live cells. Abnormal values may also indicate some cellular stress to process conditions, defective media ingredients or contamination.
  - Viable Cell Density (VCD): Is an important parameter used to quantify the density of viable cells in the culture. The VCD indicates the viability of the cells and a reduction in VCD can indicate cellular stress, inadequate nutrients or contamination.
  - Doubling Time (DT): This parameter describes the proliferative capacity of the cells. An increase in DT can indicate cellular stress, inadequate nutrients or contamination.
  - Cell phenotype: visual inspections under the microscope can reveal unusual morphological changes and/ lytic activity.
  - Assessment of pluripotency and self-renewal: one of the main characteristics of ESC is their pluripotent nature and ability to self-renew. The transcriptomic expression of genes [redacted]

([redacted] is assessed to confirm that the cells have not differentiated and maintain pluripotency.

- Microbiological quality and adventitious agents:
  - *Mycoplasma* spp.: although these organisms are not typically recognized as foodborne pathogens, they are an important concern in cell culturing environments. Unlike other bacteria, they exhibit a particularly slow growth rate which can prevent early detection. Additionally, they can evade detection due to their small size and they can resist common antibiotics due to their lack of cell walls. Mycoplasmas can induce various responses in cells such as changes in cell metabolism, growth characteristics, and gene expression (Harasawa et al., 1993, Uphoff and Drexler, 2014). The genus does contain human pathogens, but *Mycoplasma* spp. infections via oral consumption are not commonly recognized as a significant route of transmission as they are primarily known to infect the respiratory and urogenital tracts of humans and animals, and their transmission is typically through respiratory droplets or close physical contact (Taylor-Robinson, 1996, Waites et al., 2012). *Mycoplasma* spp. can be introduced via media ingredients, manipulation, or cross-contamination with other cell lines. Therefore, Mycoplasma detection is performed as one of the release testing assays of the MCB and WCB.
  - *Mycobacterium* spp.: *Mycobacterium* spp. organisms are not typically recognized as foodborne pathogens but are an important concern in cell culturing environments. Similarly to *Mycoplasma* spp., these microorganisms are small and exhibit slow growth. The primary concerns with *Mycobacterium* spp. relate to their role in diseases such as tuberculosis and leprosy, and their environmental persistence, rather than transmission through food consumption (Falkinham, 2009) Their presence in food is more often an indicator of environmental contamination as they have been found in water, soil, and biofilms (Hamilton et al., 2017). It is noted that some studies have detected *Mycobacterium* spp. in dairy products and meat, indicating that foodborne exposure, while not a primary transmission route, could be possible (Sevilla et al., 2017). This possible risk is notably higher with the consumption of unpasteurized milk or inadequately cooked meat as these organisms have shown resilience against food safety treatments such as pasteurization and other heat treatments (Collins, 1997). Therefore, *Mycobacterium* spp. detection is performed as one of the release testing assays of the MCB.
  - Sterility – viable microorganisms (bacteria, mould, and yeast sterility test): these contaminants can be introduced during MCB manufacture. Unlike *Mycoplasma* spp., these will typically be visible within 24-72h (depending on the species) after being introduced into the cell culture. Sterility testing is an indicator of the aseptic conditions and sterility of the process. However, contamination is easily detected via visual inspection (e.g., turbidity of the medium, appearance of small aggregates, distinct odour) and a decrease in growth performance indicators is also indicative of contamination.
  - Viruses: Since the cells have been exposed to components of bovine, murine and porcine origin during the stages of isolation and adaptation, the presence of potential adventitious agents from these species has been tested. The selection and methodology for the detection of porcine and bovine viruses was performed following ICH Q5A (R2). Murine Leukaemia virus was tested for in the MCB due to the use of murine embryonic fibroblasts in the early isolation process. This virus is routinely monitored in laboratories working with murine cells as it is a widespread adventitious agent in this species. Chicken and duck viruses were selected based on prevalence and importance in the poultry sector. It is worth noting that most animal viruses do not represent a hazard as they are unable to cross the species barrier (tropism) (Zai and Yuan, 2024). Retroviruses are among the most persistent and common families that can be found in avian and other animal species due to their evolutionary role. Retroviruses are commonly integrated into the genome, but most are non-infectious and only produce defective particles unable to infect other cells and reproduce. However, they are also overly sensitive to process conditions and heat. Additionally, a non-targeted transcriptomic analysis using NGS (Next Generation Sequencing) was performed to detect the potential presence of replicative viruses.

The MCB was released based on the following criteria:

- Analyses showed no microbial growth and an absence of adventitious agents.
- The species was confirmed as *Anas platyrhynchos*.
- Doubling time and viability were in accordance with the specifications at the banking and at thawing.
- The cells were pluripotent.

Based on the analyses performed, cells from the MCB were confirmed to be from *Anas platyrhynchos* and were not contaminated with other species. The cells were free from microbial contamination and adventitious agents and met the specifications for pluripotency (Table 4). The certificates of analysis from the analytical results can be found in Confidential Appendix 6a - Master Cell Bank Results. Results regarding cell viability, recovery, density and proliferation were performed in-house and records maintained.

Table 4. Characterization and Qualification of the Master Cell Bank (MCB)

Attribute	Category	Assay / Reference Methods	Specification	Internal or external	Result (Confidential Appendix 6a)
Sterility (bioburden)	Safety	Sterility test and Qualification of Test Article by Direct Inoculation method EP 2.6.1. USP 71.	Negative (in the limit of referenced method)	External	**Negative**
Mycoplasma detection	Safety	Test for *Mycoplasma* spp. (incl. Mycoplasmastasis assay) EP 2.6.7., USP 63	Negative (in the limit of referenced method)	External	**Negative**
Mycobacteria	Safety	Mycobacteria detection by direct culture Eur Pharmacopoeia 2.6.2	Negative (in the limit of referenced method)	External	**Negative**
Adventitious agents/ viruses[1]	Safety	Internal PCR method from third party, compliant with EP 2.6.21	Negative (in the limit of referenced method)	External	**Negative**
Adventitious agents / viruses	Safety	Agnostic research of adventitious viruses in Duck Cell Banks by transcriptomic approach (Next-Generation Sequencing or NGS) ICH Q5A(R2)	No detection of human and animal pathogenic viruses	External	**No replicative viruses detected**
Identity Confirmation	Purity / identity	EP 2.6.21[2]	*Anas platyrhynchos* species confirmation and absence of cross-species contamination	External	***A. platyrhynchos*. No other species detected**
RT-qPCR pluripotency	Purity / identity	Internal Standard Operating Procedure: SOP-MOLB-010: Internal RT-qPCR method	Cells express (fold change = [10: ∞]) genes *Anas platyrhynchos* [redacted] compared to the negative control (fibroblasts)	Internal	**Pass**
Cell Recovery	Quality	Internal Work Instructions: WI-PDEV-002: Thawing of Duck WI-PDEV-001: Seed Train Culture and Amplification of Duck Suspension Cells WI-PDEV-005: Cell counting methods with the Norma XS	Cell Viability >50% at thawing Cell Viability >=75% after three days of recovery	Internal	**Pass**

Table 4. Characterization and Qualification of the Master Cell Bank (MCB)					
Attribute	**Category**	**Assay / Reference Methods**	**Specification**	**Internal or external**	**Result (Confidential Appendix 6a)**
Cell Density	Quality	Internal Work Instructions: WI-PDEV-002: Thawing of Duck Suspension Cells WI-PDEV-005: Cell counting methods with the Norma XS	Viable Cell density > 1E6 C/mL at thawing	Internal	**Pass**
Cell proliferation	Quality	Internal Work Instructions: WI-PDEV-001: Seed Train Culture and Amplification of Duck Suspension Cells WI-PDEV-005 Cell counting methods with the Norma XS	Doubling time 15h-25h for 3 consecutive passages observed within 3 to 5 weeks post-thaw	Internal	**Pass**

Note: PCR = Polymerase Chain Reaction; RT-qPCR = RT-qPCR = Quantitative Reverse Transcription Polymerase Chain Reaction; USP = United States Pharmacopeia; NGS = Next Generation Sequencing; EP = European Pharmacopoeia

[1]Adventitious agents: the full list of species tested by PCR can be found in pages 6 and 7 of Confidential Appendix 6a - Master Cell Bank Results

[2]Species tested in the quantitative PCR: *Homo sapiens*, *Chinese hamster*, *Mus musculus (mouse)*, *Rattus norvegicus (rat)*, African green monkey, *Canis familiaris (dog)*, *Felis catus (cat)*, *Oryctolagus cuniculus (rabbit)*, *Equus caballus (horse)*, *Gallus gallus (chicken)*, *Sus scrofa (pig)*, *Bos taurus (beef)*, *Spodoptera frugiperda (fall armyworm)*, *Anas Platyrhynchos (duck)*.

B.4.2.4. Creation and validation of the working cell bank

The Working Cell Bank (WCB) is a bank derived from cells from the qualified MCB which were amplified and then cryopreserved following the same process as the MCB. Food-safe medium was used to create the WCB. Whilst the MCB is meant as a back-up for the production of the WCB, the WCB is the starting point of cell biomass production. Less stringent testing is required for the WCB as the cells are directly sourced from the qualified and fully characterized cells from the MCB. Consequently, only cell phenotype parameters were evaluated along with sterility and *Mycoplasma* detection which could have been introduced during the manufacture of the WCB.

The WCB was released based on the following criteria:

- Analyses showed no microbial growth and an absence of *Mycoplasma* spp.
- The species was confirmed as *Anas platyrhynchos*.
- Doubling time and viability were in accordance with the specifications at the banking and at thawing.
- The cells were pluripotent.

The results show that cells from the WCB were duck (*Anas platyrhynchos*) and were free from contamination (Table 5). The certificates of analysis from the analyses are presented in Confidential Appendix 7 – Working Cell Bank Results. Results regarding cell viability, recovery, density and proliferation were performed in house (Confidential Appendix 9 - Growth Stability Report).

Table 5. Characterization and qualification of the Working Cell Bank (WCB)						
Attribute	**Category**	**Assay / Reference Methods**	**Specification**	**Internal or external**	**Result**	**Appendix**
Sterility (bioburden)	Safety	Sterility Testing EP 2.6.1. USP71.	Negative (in the limit of referenced method)	External	**Negative**	Confidential Appendix 7
Mycoplasma detection	Safety	Quantitative PCR Eur Pharmacopoeia §2.6.7	Negative (in the limit of referenced method)	External	**Negative**	

Table 5. Characterization and qualification of the Working Cell Bank (WCB)						
Attribute	**Category**	**Assay / Reference Methods**	**Specification**	**Internal or external**	**Result**	**Appendix**
Identity Confirmation	Purity / identity	Internal Quantitative PCR method from third party[1]	*Anas platyrhynchos* species confirmation and absence of cross-species contamination	External	***A. platyrhynchos*. No other species detected**	
RT-qPCR pluripotency	Purity / identity	Internal Standard Operating Procedure: SOP-MOLB-010: Internal RT-qPCR method	Cells express (fold change = [10: ∞]) genes *Anas platyrhynchos* [redacted] compared to the negative control (fibroblasts)	Internal	**Pass**	
Cell Recovery	Quality	Internal Work Instructions: WI-PDEV-002: Thawing of Duck cell line WI-PDEV-001: Seed Train Culture and Amplification of Duck Suspension Cells WI-PDEV-005: Cell counting methods with the Norma XS	Cell Viability >50% at thawing Cell Viability >=75% after three days of recovery after thawing	Internal	**Pass**	Confidential Appendix 7
Cell Density	Quality	Internal Work Instructions: WI-PDEV-002: Thawing of Duck Suspension Cells WI-PDEV-005: Cell counting methods with the Norma XS	Viable Cell density > 1E6 C/mL at thawing Viable Cell density > 1.5E6 C/mL at thawing Cell density targeted for vials at banking = 20E6 C/mL (the density at which the cells are initially banked)	Internal	**Pass**	
Cell proliferation	Quality	Internal Work Instructions: WI-PDEV-001: Seed Train Culture and Amplification of Duck Suspension Cells WI-PDEV-005 Cell counting methods with the Norma XS	Doubling time 15h-25h for 3 consecutive passages observed within, 3 to 5 weeks post-thaw	Internal	**Pass**	

Note: PCR = Polymerase Chain Reaction; RT-qPCR = Quantitative Reverse Transcription Polymerase Chain Reaction; USP = United States Pharmacopeia; CFR = Code of Federal Regulation, EP = European Pharmacopoeia.

[1]Species tested in the Quantitative PCR: *Homo sapiens*, *Chinese hamster*, *Mus musculus (mouse)*, *Rattus norvegicus (rat)*, African green monkey, *Canis familiaris (dog)*, *Felis catus (cat)*, *Oryctolagus cuniculus (rabbit)*, *Equus caballus (horse)*, *Gallus gallus (chicken)*, *Sus scrofa (pig)*, *Bos taurus (beef)*, *Spodoptera frugiperda (fall armyworm)*, *Anas Platyrhynchos (duck)*.

B.4.3. Production process description

Gourmey's process starts with the isolation of ESCs from fertilized duck eggs. Briefly, the cells are expanded continuously in different media conditions until they exhibit the desired phenotypic and sterility characteristics and are adapted to fully characterized food-safe culture medium. At this point, the cell line development is completed, and this concludes the research phase. Cells are then moved on to the next steps of the manufacturing process, which include the creation of established cell banks, growth in the bioreactor and harvesting for additional processing. A standard overview of the production process can be found below.

The production process is comprised of the following broad phases:

1. Reception of raw materials
2. Preparation of culture medium
3. WCB vial thaw and recovery
4. Continuous culture to expand cells in shake flasks and N-1 Suspension Bioreactor (SBN-1)

5. Seeding a larger suspension bioreactor N Suspension Bioreactor (SBN) amplify the cells
6. Biomass harvest - wash and concentration
7. Biomass storage - blast freeze and storage.

With each scaling step, Gourmey will perform a new hazard analysis and update the Food Safety Plan to identify and control any new food safety hazards that may arise from scaling up. Moreover, Gourmey will ensure that the cell cultured duck meets the same specifications outlined in this dossier.

B.4.3.1. Reception and storage of raw materials

Raw materials and ingredients including growth media components, reagents, food contact materials, among others are received, inspected and stored at Gourmey's facilities.

The ingredients, raw materials and consumables are purchased from approved suppliers following Gourmey's Supplier Management Program. All ingredients are stored according to the conditions indicated by the supplier.

B.4.3.2. Preparation of culture medium

For biomass production, a chemically defined food-safe medium is used (Confidential Appendix 5a - Media Composition & Risk Assessment and Confidential Appendix 5b – Extensive Media Component Risk Assessment). These annexes contain a list of the raw materials used in the manufacturing process of the cell cultured duck along with their functional role and regulatory status in Australia & New Zealand. Although some of these media ingredients are permitted food additives, their use in Gourmey´s production process is not to perform the same technological function as the approved food additive use, and the use of these substances are inputs in the downstream production process, and they do not perform a technological function in the final cell cultured duck. The culture medium is manufactured and stored prepared until it is ready for use in production. During the production process, the cells are continuously replenished with fresh media from the same batch of prepared media as required until the media is used up. One batch of medium can be used for multiple separate harvests with continuous passaging of the cells.

All media used during the production of the batches of cell cultured duck presented in this dossier were prepared using water for injection and were sterile filtered using a single-use filter (≤0.22µm). Media preparation was performed under appropriate hygienic conditions in a separate designated room. Samples are taken to measure pH and osmolarity during media preparation to ensure operational parameters are met. Osmolarity adjustments are made by additional mixing or the addition of NaCl. pH adjustments made as necessary using sodium hydroxide. The prepared media was labelled with a unique identifying code and date of preparation and refrigerated until use.

At commercial scale production, treated and softened potable water from the municipal public supply will be used. Adequate preventive controls will be in place to ensure that the water is not a source of contamination.

B.4.3.3. Cell cultured duck production (cell expansion and biomass production)

As described in B.4.1, the production process is semi-continuous as multiple independent harvests of the cell cultured duck are possible from a single WCB vial.

The cell cultured duck is derived from a single vial taken from a qualified WCB. A seed train is established, and the cells are routinely passaged and subjected to a number of proliferation and expansion steps to increase the volume of cells at difference stages of scale:

- Shake flasks – Are used to proliferate and expand the cells from a WCB vial until there is sufficient viable cell density (VCD) to pool the cells and seed the intermediate sized bioreactor (SBN-1). A small volume of the pooled cells is retained from the shake flasks and kept in culture parallel to the SBN-1. These shake flasks can be used as a new starting point for a subsequent independent harvest through the maintenance of a continuous seed train to seed the intermediate sized bioreactor (SBN-1). Cells are continuously passaged during the production run and culture medium is replenished.
- Suspension bioreactor SBN-1 – Provides a continuous seed train in an intermediate sized bioreactor

to expand the volume to the cells used to seed the largest volume bioreactor. One or more SBN-1 can be in operation in parallel to seed the SBN. When the process is ready to seed the suspension bioreactor (SBN), a portion of the remaining cells is retained as part of Gourmey's semi-continuous process and used to maintain a continuous seed train in the SBN-1 bioreactor once it is replenished with culture media. This continuous seed train in the SBN-1 allows for the operation to seed and harvest the SBN bioreactor more frequently and reduces the frequency needed to start the process from a WCB vial. Cells are continuously passaged during the production run and culture medium is replenished.

- Suspension bioreactor SBN - The largest volume bioreactor (SBN) is seeded, and the cells proliferate and expand until they reach the desired VCD before harvest. SBN is seeded multiple times from cells taken from SBN-1 to yield independent batches of cell cultured duck.

Cell proliferation is defined increase in cell numbers resulting from cell division (Yang et al., 2014). During cell proliferation, cells divide to produce two daughter cells when placed under favourable conditions for growth, thus amplifying the number of cells in a given culture. Cell proliferation and expansion is essential for the production of the cell cultured duck and is influenced by the concentration of certain nutrients and by-products from cell metabolism. Thus, the performance of the cells is monitored and controlled at each stage.

The cell performance indicators and rationale are discussed in Section B.4.2.2. Additionally, other key performance indicators are monitored during the process (Table 6). Confidential Appendix 24 – process parameters and operational limits includes the process parameters and operational limits for each of these indicators.

Temperature, dissolved oxygen, pH are environmental conditions that influence cell health and metabolism. Cells are highly sensitive to temperature variations; it is therefore crucial to monitor for optimal cellular metabolism. Dissolved oxygen is essential for cellular respiration and maintaining the appropriate level of dissolved oxygen is important for efficient cell metabolic processes. The pH level affects enzyme activities and the overall biochemical reactions within the cells.

In the bioreactors, agitation is important to monitor as it is used to ensure uniform distribution of cells, nutrients, and gasses (like oxygen) throughout the culture. Proper agitation helps prevent cell sedimentation and clumping and ensures that all cells have equal access to the necessary components for growth. However, excessive agitation can cause shear stress, damaging the cells.

Glucose and L-glutamine provide energy to the cells and aid proliferation. These levels are monitored in the shake flask and SBN-1 stages and are monitored and adjusted in the SBN to maintain the optimum requirements for growth.

Cell metabolism converts glucose to pyruvate and lactate via glycolysis. Thus, lactate is an important indicator of metabolic conditions. Increased levels of lactate can indicate metabolic stress and lead to a reduction in the pH. The pH needs to be kept stable as it affects overall cell health (viability, viable cell density (VCD), doubling time (DT) and morphology) and metabolism. Further, glutamine is converted to glutamate and ammonia, which are also monitored.

Monitoring the consumption rate of glucose and glutamine and the production of metabolites provides valuable data on the performance of the cells.

The cell performance indicators that are routinely monitored are presented in Table 6. More specific details on process parameters and operational limits limits are provided in Confidential Appendix 24 - Process parameters and operational limits.

Table 6. Monitored Cell Performance Indicator Values at Each Stage of Biomass Production				
Parameters	**Shake Flask**	**SBN-1**	**SBN**	**Rationale**
Viability on thawing	Monitored	N/A	N/A	Indicator of cell health
Cell Viability	Monitored	Monitored	Monitored	Indicator of cell health and process performance
Viable Cell Density	Monitored	Monitored	Monitored	Indicator of cell health and process performance
Doubling time	Monitored	Monitored	Monitored	Indicator of cell health and process performance
Cell diameter	Monitored	Monitored	Monitored	Indicator of cell health and process performance
Cell Imaging, Morphology	Monitored	Monitored	Monitored	Indicator of cell health and process performance
Cell Imaging, Contamination check	Monitored	Monitored	Monitored	Absence of obvious biological contaminants
Temperature	Monitored and controlled	Monitored and controlled	Monitored and controlled	Indicator of process conditions
Dissolved oxygen	N/A	Monitored and controlled	Monitored and controlled	Indicator of culture environment and process performance
pH	N/A	Monitored and controlled	Monitored and controlled	Indicator of culture environment and process performance
$\%CO_2$	Monitored and controlled	Monitored and controlled	Monitored and controlled	Indicator of culture environment and process performance
$\%O_2$	N/A	Monitored and controlled	Monitored and controlled	Indicator of culture environment and process performance
Agitation	Monitored and controlled	Monitored and controlled	Monitored and controlled	Process parameter affecting mixing and gassing
Glucose	Monitored	Monitored	Monitored and controlled	Indicator of cell health and media addition indicator for SBN
Glutamine	Monitored	Monitored	Monitored and controlled	Nutrient source and cell metabolism monitoring and media addition indicator for SBN
Osmolality	Monitored	Monitored	Monitored	Indicator of culture environment affecting cell health
Notes: SBN-1 = Suspension bioreactor N-1 (smaller volume), SBN = Suspension bioreactor (larger volume); N/A = Not Applicable				

Sterile weldable tubes, hoses and other materials are used in the biomass production process. Some of these materials are sterilized and reused, whilst others are single-use and are disposed of after each operation.

Gasses and carbon dioxide introduced into the bioreactor are filtered at 0.22 µm to mitigate the risk of introducing potential microbial contamination.

B.4.3.3.1. Vial thaw and initial recovery

The process to produce cell cultured duck starts by thawing a vial from the WCB. The contents of the vial are thawed, centrifuged to remove the freezing media, and re-suspended in food-safe culture medium (FSCM) where the viability upon re-suspension is measured (>50% cell viability). The cells are then inoculated into a shake flask and cell density, doubling time, viability and viable cell density (VCD) are monitored. Depending on the cell density, additional FSCM may be added to provide necessary nutrients for cell recovery. Once the cells have recovered, they are used to start the seed train. Samples of the supernatant are retained to perform sterility testing. Cell growth indicators and VCD are monitored throughout the process.

B.4.3.3.2. Continuous culture in shake flasks (seed train)

The seed train is continuously cultured in shake flasks throughout the entire production process. Every 2-3 days, shake flasks are monitored and a cell passage is performed. During passaging, FSCM is added to bring the cell culture to an optimal cell density for continued culturing. Depending on the final cell culture volume, the cell culture may be transferred to one or multiple larger shake flasks to increase the overall culture volume. VCD and cell counts are regularly performed to assess the performance of the cells. Immediately before the inoculation of the SBN-1 bioreactor, samples are taken to confirm the pluripotency of the cells and *Mycoplasma* spp., detection is performed. Visual inspection of the cells via microscopy (maximum x40 magnification) is routinely performed to monitor phenotype (morphology) and cell culture process indicators are analysed including pH, gasses, osmolarity, and chemical parameters. Cells from the shake flasks can be used to restart the process in the event of contamination in the bioreactors. Continuous culture in the shake flasks is maintained until the final harvest.

B.4.3.3.3. Continuous culture in SBN-1

Cells from the shake flask(s) are used to inoculate the N-1 suspension bioreactor (SBN-1) which contains FSCM to target an optimal cell density for culturing. Every 2-3 days, the cell culture in the SBN-1 is monitored and a cell passage is performed. During cell passaging, FSCM is added to bring cell culture to an optimal cell density. Before inoculating the larger bioreactor (N suspension bioreactor or SBN), samples are taken to test for sterility, expression of pluripotency markers and *Mycoplasma* spp. Visual inspections of the cells (under the microscope) and cell culture process indicators are analysed including pH, gasses, osmolarity, and chemical parameters. The continuous culture in the SBN-1 is maintained until the final harvest.

B.4.3.3.4. SBN inoculation and expansion

Cells from SBN-1 are used to inoculate the larger SBN bioreactor which is pre-filled with FSCM to target an optimal cell density. The viability of the cells and VCD are monitored daily. Similarly, to the previous steps, inspections under the microscope, cell counting and monitoring of certain metabolites are performed throughout the expansion in the SBN. Once an optimal cell density and a sufficient volume are achieved (usually within 4-5 days), the cells are harvested. Before harvesting, samples are taken to test for sterility, pluripotency markers and *Mycoplasma* spp., detection.

B.4.3.3.5. Cell cultured duck harvest, washing and packing

The cells are transferred from the SBN into a centrifuge where they are concentrated by removing the spent medium. Following cell concentration, saline solution (0.60%) is added to the centrifuge to wash the biomass. The washed biomass is finally transferred to food-safe bags, which are vacuum packed and frozen at -20±1°C (-4°F) or less.

Although Gourmey has performed a risk assessment on the media and considers it to be food-safe, washing the biomass is a key precautionary step adopted to reduce carry over of the media ingredients ethanolamine, pluronic acid and recombinant bovine insulin which have limited or no history of use in food to the final product. The concentration of each of these components was analysed in five batches of cell cultured duck biomass and the results can be found in Section B.5.

B.4.3.3.6. Storage

The cell cultured duck biomass is frozen at -20°C (-4ºF) or less until it is required for further processing. Freezing ensures the quality of the biomass is maintained by inhibiting potential enzymatic reactions and the growth of microorganisms that may affect the shelf life of the product.

B.4.3.3.7. Final Product Production

The cells will be combined with other suitable food ingredients, but the processing technologies and final formulations of finished products are not discussed further in this dossier.

B.4.4. Summary Food Safety Management

Gourmey has carried out an assessment on the potential physical, microbiological, chemical and radiological hazards in the production process and has applied adequate measures for each identified risk. In-process controls and monitoring measures have been implemented to demonstrate the effectiveness in reducing and/or eliminating identified risks. Confidential Appendix 3 - Food Safety Plan contains further details on the measures in place based on the HACCP and Critical Control Points principles from the Codex Alimentarius (CXC1-1969, 2022) and Standard 3.2.1 - Food Safety Programs (FSANZ, 2023a)

The design and implementation of the monitoring plan has been carried out by qualified individuals and an interdisciplinary team of trained professionals from different areas of expertise. All Gourmey's staff have received general food safety training as well as detailed training tailored to their specific role and activities. Secure copies and/or original programs, SOPs and instructions have been made available and accessible to all members at all times.

Gourmey has identified the following Preventive Controls:

- **Supply-chain preventive controls / Supplier Management**: the safety of the raw and source materials is key to guaranteeing the safety of the process and final product. Consequently, Gourmey has developed and implemented processes to ensure key materials are sourced from approved suppliers and that their performance is monitored. Before purchasing ingredients from new suppliers, Gourmey will conduct due diligence activities by requesting various documents pertaining to business, operations, and quality practices from all its potential suppliers to aid in the evaluation of the supplier and their supplied materials. Various quality information is also needed to determine if the supplier uses processes and procedures that provide the same level of public health protection as those required under the preventive control provisions of EU legislation. This is to ensure that the materials or ingredients, sourced from suppliers that typically work with biologics or pharmaceutical clients would not be considered adulterated or misbranded in the context of food. The information requested by Gourmey from suppliers used for its evaluation may be collected directly from the supplier via email, questionnaires/surveys, and or through specific documentation requests. Gourmey uses various supplier verification activities for its suppliers. They include, but are not limited to, reviewing of supplier and food safety policies and procedures, product sampling and testing, receiving inspections, and or onsite auditing by Gourmey or a 3rd-party (wherever practical).

  Sanitation: Safety is largely determined by the hygienic condition of surfaces, equipment and areas. Gourmey applies specific sanitation standard operating procedures (SSOPs) and hygienic zoning, to classify areas based on risk for the product, as sanitation preventive controls.

  SOPs applicable to food-contact equipment and process areas where there is exposed product or cells, where a subsequent process preventive control does not exist to eliminate a pathogen are listed as relevant sanitation preventive controls. The hygienic zoning within the Gourmey production facility plays a critical role in reducing the risk of product contamination with environmental pathogens. This is particularly vital in areas of the bioprocess or non-sterile production activities where there are no subsequent controls to eliminate or remove such hazards. Implementing effective hygienic zoning is essential to maintain the integrity and safety of the products in these exposed areas.

Environmental monitoring of the production area and relevant laboratories for pathogens such as *Listeria spp*. and *Salmonella spp.* is used to verify the effectiveness of sanitation procedures and hygienic zoning strategies. These verification activities are used to assure the safety of the processing environment against contamination of products from environmental pathogens.

- **Aseptic techniques**: In the context of cultured meat and cell cultured products for human consumption, in the absence of any controls in place, the application of aseptic procedures, practices, and processes are consistent with the current scientific understanding of safe food manufacturing for these product types and therefore applicable as preventive controls. The aseptic preventive controls are intended to significantly minimize the risk of biological food safety hazards and other general biological contaminants that may be introduced to the cell culturing process. Different measures have been implemented through the training of staff on aseptic handling techniques which also include techniques on sterile welding of transfer tubes and autoclaving. Verification activities of aseptic practices are incorporated at various stages in the process such as in-house sterility testing at different points of the process and qualification of cell banks. Media is also filtered when it is prepared internally to ensure a sterile environment is maintained when introduced to the cells.

- **Process preventive controls**: The final harvested product is washed with saline solution prior to packaging to mitigate the risk of the potential presence of metabolites and non-consumable components from the media used at levels that could represent a food safety hazard. As previously mentioned, Media is also filtered when it is prepared internally to ensure a sterile environment is maintained when introduced to the cells. All harvested lots are sampled and are tested for the minimum required microbiological safety verification assays. Gourmey utilizes a hold and positive release strategy for all its products to ensure no product is shipped to customers until product has been verified as clearing all required safety specifications.

- **Allergens**: No specific allergen preventive controls have been identified by this plan to control risk of allergens that are not able to be controlled by either Supplier Preventive Controls, Sanitation Preventive Controls, or other GMPs. The risk for cross contamination is considered low and not reasonably likely to occur in an aseptic controlled environment. All materials that are introduced into the facilities must be first approved and their documentation is reviewed as part of the supplier and material programs, which include identification of risks from any potential major allergens where applicable. As part of this novel food safety dossier, the allergenicity of the product itself (cell cultured duck protein) has been considered as well as allergenicity to media components and media ingredients such as recombinant proteins. Also considered is the potential for cross-contact of egg allergen proteins carried over from isolations. This has been found to be low risk to the consumer and not reasonably likely to occur with the other established preventive controls, prerequisite programs, and GMPs in place. There are no known major food-borne allergens used by Gourmey in the production of this product or present in the processing facility. If, in the future, any known allergens are required for use, the Food Safety Plan will be re-evaluated to ensure appropriate controls are implemented. Any allergens maintained will be appropriately labelled.

Traceability also plays a major role in Gourmey's quality and food safety systems. As the identity, source, and history of the cell line used to produce Gourmey's cell cultured duck is critical to assure the safety and authenticity, it marks the beginning of the scope of Gourmey's traceability system. Identification of the egg laying flock's strain, batch code, and date of delivery to the lab where cell isolation is obtained from the egg supplier. The unique identification codes assigned to the cell line during its development are recorded to ensure that whenever a new ID is assigned during cell banking, the previous unique identifying code is noted to ensure it can be traced back to its source material. Gourmey assigns unique identifying codes for each cell line, research cell bank, master cell bank, and working cell bank created to ensure traceability when they are used. Batch records are kept tracing all raw materials used in the process along with information about the date, time and involved operators. More details can be found in Confidential Appendix 3 - Food Safety Plan.

A summary of the identified risks can be found in Table 7 below along with the preventive/control measures.

Table 7. Simplified list of risk and control and preventive measures identified for each process step		
Phase	**Risks**	**Control/Preventive measures**
Isolation	Cell identity; contamination with bacteria, mould and/or yeast from the environment, reagents or eggs; residues from veterinary medicines	Application of GCCP, cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Cell line establishment and adaptation	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents	Application of GCCP, cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Creation and validation of an MCB	Cell identity; contamination with bacteria, mould and/or yeast from the environment, reagents or eggs; residues from veterinary medicines	Application of GCCP, cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Creation and validation of a WCB	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents	Application of GCCP, cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Media preparation	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents	Application of cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Media filtration; Sanitation Program
Biomass production	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents	Application of cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Harvest and washing	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents; residues from cleaning chemicals; presence of media residues above safe limits	Application of cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Sanitation Program
Storage	Cell identity; contamination with bacteria, mould and/or yeast from the environment or reagents	Application of cGMP and aseptic environment; Environmental monitoring; Supplier Management Program; Temperature control

As previously mentioned, cells are closely monitored during each step of the process. A decrease in viability and/or cell density, pH may be the first indicators of contamination as well as peaks in oxygen consumption, bad odour or increased turbidity in the bioreactor. Although these parameters are not safety-related, they do provide important information about the health of the culture and, therefore, contribute to maintaining a safe and stable production.

All materials and consumables that come into contact with the duck cells have been evaluated to ensure that the materials are approved for use in food. However, most of the auxiliary materials, tubing and others are produced by companies specialised in the manufacture of medical devices and materials for contact with pharmaceuticals and therapeutics. Consequently, companies in the space are not yet familiar with the food-specific requirements for producing plastics and polymers for direct or indirect contact with food. However, they do comply with more stringent rules as the testing required for materials in contact with injectables and other substances is tightly regulated (USP Class VI plastic material). As such, it is reasonable to expect that these manufacturers will become food contact producers in the future once the space and technology have been sufficiently developed to be seen as a potential new market for these products.

As confirmed by the analyses performed in five independent batches of cell cultured duck Confidential Appendix 8 Composition and Purity, plastic residues were not detected in the final product. Polymers used in the process can therefore be deemed safe. The risk of plastics being present in the final product is a horizontal risk in the food industry and hence the same level of risk should be perceived for cultured food products.

The polymers in direct or indirect contact with the cells in the production process are:

- Ultra-low-density and low-density polyethylene (approved under US 21 CFR §177.1520);
- Polyethylene (approved under US 21 CFR §177.1520);
- Ethylene-vinyl acetate (approved under US 21 CFR §177.1350);
- Polyethersulfone (approved under US 21 CFR §177.2440).

The applicant highlights that the food contact polymers comply with US regulations, aligning with Australian Standard AS 2070-1999 for plastics materials used in food contact (AS 2070:1999).

B.5. Information on the impurity profile for a typical preparation

Five batches of the cell cultured duck that were representative of the production process outlined in Section B.4. were analysed for the presence of impurities. The parameters in Table 8 were selected to give a comprehensive overview of the potential contaminants that could be introduced during the production process. All analyses were performed using internationally recognized methods or adequately validated methods at accredited laboratories. The CoAs are provided in Confidential Appendix 8 Composition and Purity.

The panel of impurity tests selected was based on an extensive risk assessment. The rationale for each is presented below:

- **Microbiological parameters (total aerobic count, yeast and moulds, enterobacteria, *Salmonella* spp., *Listeria monocytogenes, Campylobacter* spp.)** are tested to ensure that no contamination with bacteria, fungi or mycoplasma was introduced during cell expansion and harvesting. They are indicators of good hygiene practices and microbiological safety of the process.

- **Heavy metals** (cadmium, lead, arsenic, and mercury) are tested following the current regulations on food contaminants.

- **Dioxins and PCBs** are monitored as these contaminants are widely present within and outside the food chain and are of particular concern in meat and fish products.

- **Residues of culture media ingredients and cell metabolites** that need monitoring because of the risk analysis performed, mainly pluronic acid, ethanolamine and bovine recombinant insulin, which have limited or no history of use in food. By-products from cell metabolism such as organic acids and biogenic amines were analysed as they can also provide insights into the metabolic state of the cells, offering valuable information for optimizing cell culture conditions and processes.

- **Other contaminants**: The presence of polymer residues (plastics) was evaluated as the cells are continuously in contact with polymers throughout their cultivation. All polymers are approved food contact materials under the US legislation.

- **Antibiotic and veterinary medicine residues** are highly unlikely to be present in the biomass. Antibiotics are only used in the early stages if cell isolation. The cells are continuously washed during subsequent passages. The theoretical dilution is in the magnitude of x10,000 times based on the number of passages and volumes handled. Veterinary medicines can only be introduced via the source material (egg) and will also be eliminated through continuous passaging. Antibiotics and veterinary medicine residues were tested as there are internationally recognised methods and standardised screening panels available in multiple food matrices. Moreover, there are specific maximum residue levels for veterinary drugs set for food whereas there are no specific limits set for growth factors used as media inputs.

The analytical data summarised in Table 8 show that the biomass was free from pathogens and has negligible levels of food contaminants and media residues. Regulated food impurities such as lead, mercury and cadmium were not detected in the biomass (below the analytical limits) and in compliance with the existing legal limits for the maximum levels of contaminants and natural toxicants as per Schedule 19 (FSANZ, 2024c)

along with permitted levels of dioxins and PCBs. The data below show the absence of plastics, antibiotics and veterinary medicine residues in the five batches tested.

The cell cultured duck is tested for general microbiological indicators, aerobic plate count, Enterobacteriaceae, yeasts and moulds, pathogens *Salmonella* spp. and *Listeria monocytogenes. Salmonella* spp. and *Listeria monocytogenes* are environmental pathogens that are considered more relevant to survey in the context of a typical food processing environment once the cells have been harvested from the sterile cell culturing bioprocess. Microbiological analytical results were below the existing legal limits set for packaged heat-treated meat paste and packaged heat-treated pâté in the Australia New Zealand Food Standards Code Schedule 27 (Microbiological limits in food) (FSANZ, 2021) and in line with the Compendium of Microbiological Criteria for Food (FSANZ, 2022).

Gourmey's products adhere to a positive release protocol meaning they would not be released to market until confirmed in specification. In the event of out-of-specification results at time of production, they would not be released, and corrective actions would be taken to investigate and appropriately assign a disposition for the product.

Three batches of cell cultured duck were additionally tested for coagulase-positive Staphylococci and β-glucuronidase-positive *Escherichia coli.* Both are pathogenic and are known to cause foodborne illnesses when present in elevated counts. However, they are considered not reasonably likely to occur due to the aseptic process controls that are required for successful cell cultured duck production. As noted in Section B.4.2.3, the MCB is tested to confirm no microbial growth and an absence of adventitious agents prior to release of the bank into production. To further mitigate the risk of human pathogens introduced by employees, employee GMP controls are applied. These controls include restriction and exclusion of ill employees from production areas, no bare hand-contact with product (glove-use), and aseptic handling techniques during the bioprocess are applied to mitigate the risk of these human pathogens associated with handling. All measurements were below the limit of detection. The certificates of analysis are presented in Confidential Appendix 8.

Campylobacter spp. was tested and is included in the current specification for cell cultured duck due to its association with certain poultry product outbreaks, such as liver pâtés (CDC, 2015). Based on the risk assessment performed, *Campylobacter* spp. is not considered to be a significant food safety risk in its product as contamination is typically associated with raw poultry products during slaughter operations at farms, as well as during animal rearing processes (e.g., milking and poultry processing) (FSANZ, 2024b). Consumption of chicken meat and contact with domestic chickens were noted as imported risk factors for campylobacteriosis in Australia and New Zealand following an investigation to identify relevant risk factors for sporadic campylobacteriosis (Varrone et al., 2020). A small foodborne outbreak of campylobacteriosis was reported in Australia in 2022 with the likely cause speculated as being the inadequate cooking of duck liver reinforcing the risks posed by uncooked poultry dishes (McAllister et al., 2023).

There is a significant difference in the risk profile, origin, and handling between conventional, slaughtered poultry and cell cultured duck. The nature of the cell bank sourcing and supplier controls, including a robust cell bank acceptance criteria process, the production process, materials used, and the controls in place makes the risk of *Campylobacter* spp. contamination not reasonably likely to occur in the product. It is the intent of Gourmey to ensure final products made from cultivated duck and other approved food ingredients receive appropriate pathogen lethality using validated thermal or non-thermal processing. In the future, the intention is to remove this testing requirement from the specifications.

Biogenic amines were tested and have been assessed to have no regulatory or safety impact. The assessment of the concentration of biogenic amines can be found in Section C.2.1.3. The monitoring of these compounds provides insights into the metabolic state of the cells, offering valuable information for optimizing cell culture conditions and processes. Specifically, high levels of histamine are widely recognized for its potential to cause foodborne illness with various countries establishing regulations for maximum histamine acceptance levels in certain food products (DeBeeR et al., 2021). Histamine was not detected in the three batches analysed for impurities.

Media residue analyses for ethanolamine, insulin and pluronic acid analyses were performed in a non-accredited/certified laboratory. These tests are not routinely used in food safety and the applicant could not

identify any accredited/certified institutions that could perform these tests nor international methods for these substances. The analyses were performed in a qualified laboratory with extensive experience in the analysis of pharmaceuticals and the development of analytical methods for uncommon substances. The method utilised LC-MS-MS, which is widely used in the food industry to address food safety and authenticity. Information on the quality systems in place for control and documentation is provided in Confidential Appendix 15. The data show that levels were in line with the specifications proposed for cell cultured duck (Section B.6). Bovine insulin was below the limit of detection in all batches. Levels of pluronic acid across the five batches ranged between 177.6 and 367.9 mg/kg with an average of 292.86 mg/kg. Higher levels of ethanolamine were observed in Batches 001G, 002B and 002D compared to 001C and 001D. This variation may be attributed to several factors including mechanical disruption from harvesting procedures (centrifugation and packaging) and freeze-thaw cycles causing the physical disruption of cell membranes resulting in the gradual leakage and release of intracellular contents, including ethanolamine.

Table 8. Analysis of impurities from five batches of cell cultured duck (Confidential Appendix 8)

Impurity	Method	RB-001C	RB-001D	RB-001G	RB-002B	RB-002D	Mean values	SD
Metals								
Arsenic (mg/kg)	ICP/MS (internal method)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-
Cadmium (mg/kg)	ICP/MS (internal method)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	-
Lead (mg/kg)	ICP/MS (internal method)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-
Mercury (mg/kg)	ICP/MS (internal method)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	-
Aluminium (mg/kg)	ICP-MS, DIN EN ISO 17294-2	< 0.5	0.6	< 0.5	< 0.5	< 0.5	< 0.5	-
Barium (mg/kg)	ICP-OES (internal method)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	-
Cobalt	ICP-MS, DIN EN ISO 17294-2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-
Tin (mg/kg)	ICP/MS (internal method)	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	-
Antimony (mg/kg)	ICP/MS (internal method)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	-
Food contact materials (plastics)								
Bisphenol A (µg/kg)	LC/MS/MS (internal method)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	-
Plastics (battery of 30)	LC/MS/MS (internal method)	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	-	-
PAH (µg/kg)	GC/MS/MS (internal method)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	-
Antibiotics and veterinary medicine residues[4]								
Beta-lactams (µg/kg)	LC/MS/MS (internal method)	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	-	-
Streptomycin/amino glycoside (µg/kg)	LC/MS/MS (internal method)	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	-	-

Veterinary medicine residues (µg/kg)	LC/HRMS (internal method)	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	Not detected[3]	-	-
Aminoglycosides (µg/kg)	LC/MS/MS (internal method)	< 50	< 50	< 50	< 50	< 50	-	-
Dioxins and PCBs								
Sum of dioxins (WHO-PCDD/F-TEQ) (WHO 12) (pg/g wet weight) (medium bound)	GC/MS/MS[1]	0.00571	0.00403	0.00683	0.00544	0.00508	0.0483	0.0960
Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB TEQ) (WHO 17) (pg/g wet weight) (medium bound)	GC/MS/MS[1]	0.0120	0.0116	0.0142	0.0114	0.0095	0.0917	0.1785
Sum of dioxins and PCBs (WHO 17+ WHO 12) (PCDD/F+PCB TEQ) (pg/g wet weight) (medium bound)	GC/MS/MS[1]	0.0177	0.0156	0.0211	0.0168	0.0146	0.0578	0.0907
Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6) (pg/g wet weight) (medium bound)	GC/MS/MS[1]	0.0101	0.00714	0.0122	0.0151	0.00903	0.05169	0.0941
Biogenic amines								
Cadaverine (mg/kg)	LC/UV/DAD	< 1	< 1	< 1	< 1	< 1	-	-
Spermine (mg/kg)	LC/UV/DAD	188	191	194	174	122	173.8	29.95
Tyramine (mg/kg)	LC/UV/DAD	< 1	< 1	< 1	14.7	< 1	3.74	6.13
Tryptamine (mg/kg)	LC/UV/DAD	< 5	< 5	19.3	< 5	< 5	7.86	6.40
2-phenylethylamine (mg/kg)	LC/UV/DAD	3.32	< 1	3.82	< 1	< 1	2.03	1.42
Histamine (mg/kg)	LC/UV/DAD	< 1	< 1	< 1	< 1	< 1	-	-
Putrescine (mg/kg)	LC/UV/DAD	24.2	32.6	71.6	48.3	38.5	43.04	18.22
Spermidine (mg/kg)	LC/UV/DAD	155	171	211	174	118	165.8	33.69
Microbiological contaminants								
Total aerobic count (cfu/g)	XP V08-034	< 200	< 200	< 200	655[5]	< 10	< 200	-
Salmonella spp. (in 25 g)	BKR 23/07-10/11	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	-	-
Listeria monocytogenes (in 25 g)	BKR 23/02-11/02	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	-	-

Campylobacter spp. (in 25g)	ISO 10272-1/A1	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	-	-
Enterobacteriaceae (cfu/g)	NF V08-054	< 10	< 10	< 10	< 10	< 10	< 10	-
Yeast and mould (cfu/g)	NF V08-059	< 10	< 10	< 10	< 10	< 10	< 10	-
Media residues								
Pluronic acid (mg/kg)	LC-MS-MS (internal method)	367.9	332.3	177.6	302.8	283.7	292.86	71.86
Insulin (µg/g)	LC-MS-MS (internal method)	< 0.5	< 2	< 2	< 0.5	< 0.5	< 2	-
Ethanolamine[6] (µg/g)	LC-MS-MS (internal method)	3.0	3.1	8.5	6.8	13.3	6.94	4.28

[1]Method from Regulation (EU) No 2017/644 and Regulation (EU) No 2017/771. Values correspond to the medium-bound.
[2]Method from (Smělá et al., 2004).
[3]The list of individual residues can be found in Confidential Appendix 8.
[4]Penicillin and streptomycin used during the early stages of isolation. Antibiotic residues were below LOQ of 10 mg/kg for penicillin and 50 mg/kg for streptomycin.
[5]Data presented is the average taken for multiple data points of the batch. Four samples were tested, reporting TAC values of 1,700 cfu/g, 780 cfu/g, 140 cfu/g, and <10 cfu/g, respectively.
Notes: DAD=Diode Array Detector; ICP=Inductively coupled plasma; GC=Gas Chromatography; HRMS=High Resolution Mass Spectrometry; LC=Liquid Chromatography; PAH=Polycyclic Aromatic Hydrocarbons; MS=Mass Spectrometry; PCB=Polychlorinated biphenyls; TEQ = Tox equivalents; UV=Ultraviolet; cfu=colony forming unit; PCCD=Polychlorinated-p-dioxins; PCB28: 2,4,4'-Trichlorobiphenyl; PCB52= 2,2',5,5'-Tetrachlorobiphenyl; PCB101=2,2',4,5,5'-Pentachlorobiphenyl, PCB138=2,2',3,4,4',5'-Hexachlorobiphenyl; PCB153=2,2',4,4',5,5'-Hexachlorobiphenyl; PCB180=2,2',3,4,4',5,5'-Heptachlorobiphenyl; S= Standard Deviation; TEQ=Toxic Equivalent
[6]For ethanolamine, Batch G was one day less in culture compared to the other batches tested giving the cells less time to metabolise the ethanolamine.

Organic acids were also quantified for internal purposes in the five batches, but the results were assessed to have no regulatory or safety impact. Many organic acids, such as acetic, citric, lactic, propionic, sorbic, and benzoic- and their salts are substances that may be used as food additives in Australia/New Zealand. Furthermore, these organic acids are naturally occurring substances in all cells that are formed as metabolites during the citric acid cycle (Krebs cycle), lactic acid cycle and other physiological cycles. As such, these organic acids are widely present in all living cells and are consumed regularly as a natural and integral part of food. Moreover, organic acids are produced by bacteria in the human gut. Understanding the organic acid composition can potentially provide useful information on cell production performance. Considering the wide scope of use of many of these acids in foods, no safety concerns are anticipated from the presence of residual levels of these substances in cell cultured duck.

Table 9. Organic acid profile from five batches of cell cultured duck (Confidential Appendix 8)

Organic acid	RB-001C (mg/kg)	RB-001D (mg/kg)	RB-002B (mg/kg)	RB-002C[1] (mg/kg)	RB-002D (mg/kg)	Mean values	SD
Citric acid	< 66	< 81	134	<100	114	116	17.09
Succinic acid	< 3,040	< 3,720	3340	3,670	< 11,400	6,137	4,561
Quinic acid	< 100	< 100	< 500	<500	< 100	260	219.10
Oxalic acid	NA[2]	< 20	< 100	<100	NA[2]	100	-
Acetic acid	< 193	< 320	2400	3,040	< 417	1,952	1,368
Formic acid	98	98	< 200	<200	< 51	129.4	67.24
Pyruvic acid	< 2	< 2	4	<10	5	6.3	3.21

Table 9. Organic acid profile from five batches of cell cultured duck (Confidential Appendix 8)							
Organic acid	**RB-001C (mg/kg)**	**RB-001D (mg/kg)**	**RB-002B (mg/kg)**	**RB-002C[1] (mg/kg)**	**RB-002D (mg/kg)**	**Mean values**	**SD**
Propionic acid	<50	< 50	< 250	690	< 5	209	285.14
Malic acid	90	134	256	<125	236	168.2	73.24
Butyric acid	< 50	< 50	< 250	<250	< 50	<250	-
Pyroglutamic acid	246	305	110	<250	214	225	72.13
Glycolic acid	< 123	< 248	< 250	<250	< 200	233	28.88
Lactic acid	4,150	4,720	3780	1,750	4,690	3827.5	1,410

*SD= Standard Deviation
*SD= Standard Deviation
[1]No retain biomass was available from Batch RB-001G for the analysis of the organic acid profile. Data has been provided on alternative Batch RB-[redacted]C.
[2]NA = Not analysed due to matrix disturbances oxalic acid could not be quantified

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

B.6.1. Proposed product specification

Appropriate compositional food-grade specifications have been established for cell cultured duck based on the results of the batch analyses presented in Sections B.5 and B.6.2. Table 10 outlines the parameters and criteria that have been defined. All methods of analysis are based on internationally recognised or internally validated methods performed at accredited/certified laboratories (Confidential Appendix 13). The specifications have considered quality and food safety parameters commonly monitored in food and relevant for cultured food products.

The specifications of the cell cultured duck include the following elements:

- **Physical characteristics and composition** (e.g., proximate analysis of protein, moisture, etc.) are tested to quantify chemical parameters that may affect the formulation of products that use cell cultured duck as an ingredient. These parameters are not related to safety but define the product composition and consistency of the production process.

- **Heavy metals** (cadmium, lead, arsenic, and mercury) are tested following the current regulations on food contaminants. These specifications have been set based on the analysed levels and are similar to the maximum levels considered acceptable under Standard 1.6.1 for commonly consumed similar foods, such as meat products.

- **Residues of culture media ingredients** that need monitoring as a result of the risk analysis performed. Ethanolamine, bovine insulin and pluronic acid are the three main media residues that are monitored in the duck cells; their concentrations have been included in the specifications.

- **Microbiological parameters (total aerobic count, yeast and moulds, enterobacteria, *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp.)** are tested to ensure the microbiological quality and safety of the ingredient. Total aerobic count, Enterobacteriaceae, yeasts and moulds are included as indicators of good hygienic practices of the process. Pathogens *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. are tested specifically for safety and must not be detected.

Polymers (or plastics) have not been considered in the specifications as these are not typically included in food product specifications. The risk of these substances migrating from the material to the food is controlled and monitored at supplier level. All pertinent documentation for all food contact materials is available at Gourmey following our Supplier Management Program.

Table 10. Specification of Cell cultured duck		
Parameter	**Specification**	**Method of analysis**
Physical Characteristics and Composition		
Protein, N x 6.25	>10 %	Kjeldahl (internal method); Dumas
Moisture	>80 %	Thermogravimetry (internal method)
Protein (% dry basis)	>70 %	Calculated
Heavy metals		
Cadmium	< 0.1 ppm	ICP/MS (internal method)
Lead	< 0.1 ppm	ICP/MS (internal method)
Arsenic	< 0.1 ppm	ICP/MS (internal method)
Mercury	< 0.050 ppm	ICP/MS (internal method)
Residues		
Pluronic acid	<500 µg/g	LC-MS-MS (internal method)
Insulin	< 2 µg/g	LC-MS-MS (internal method)
Ethanolamine	< 30 µg/g	LC-MS-MS (internal method)
Microbiology		
Total aerobic count	< 3,000 cfu/g	XP V08-034[(1)]; ISO 4833
Campylobacter spp	Absence Not Detected /25 g	ISO 10272-1/A1; ISO 10272-2/A1
Salmonella spp.	Absence Not Detected /25 g	BKR 23/07-10/11; EN/ISO 6579
Listeria monocytogenes	Absence Not Detected /25 g	BKR 23/02-11/02; EN/ISO 11290-1
Enterobacteriaceae	< 100 cfu/g	NF V08-054; ISO 21528-1
Yeasts	< 100 cfu/g	TBC NF V08-059; EN ISO 21527
Moulds	< 100 cfu/g	NF V08-059; EN ISO 21527

Note: cfu = colony forming units; LC-MS-MS = Liquid Chromatography coupled with double Mass Spectrometry; ICP/MS = Inductively coupled plasma mass spectrometry

(1) In this method for total aerobic count, a spiral-plate method is used to dispense 50µl of the sample to determine a colony count. The number of colonies is counted using a counting grid supplied with the equipment and the count calculated. The LOD of the counting grid is <200 cfu/g.

B.6.2. Batch analysis

Five batches of the cell cultured duck that were representative of the production process outlined in Section B.4 were analysed for their nutritional profile. The analysis of the nutritional profile is presented in the Tables below. All nutritional analyses were performed using internationally recognised methods or validated internal methods at accredited laboratories (Confidential Appendix 13). The Certificates of Analysis (CoAs) are provided in Confidential Appendix 8**.** The data presented demonstrate that the production process is reproducible, robust and shows low variability among the different batches.

As outlined in Section B.4. Manufacturing process, the novel food is produced via a semi-continuous process where multiple independent harvests are possible from a single WCB vial. Due to the nature of the production process, completely new batches of raw materials were not used for each batch as this is not representative of how the semi-continuous production process is run. The culture medium is manufactured independent of the semi-continuous process and is stored until it is ready for use in production. During the production process, the cells are continuously replenished with fresh media from the same batch of prepared media as

required until the media batch is used up. One batch of medium can be used for multiple separate harvests with continuous passaging of the cells.

The proximate analysis of the cell cultured duck biomass is presented in Table 11. Across the five batches, the protein content ranged from 10.3-13.3 g/100g with an average of 12.03 g/100g. All batches showed compliance with the proposed specification (>10 %). In the case where multiple data points were taken for a parameter in any one batch, the average of the result obtained for each parameter in each batch has been presented below. A higher moisture value and lower protein content is observed for batch RB-002D. A higher moisture content can be attributed to residual moisture retained in the sample from the washing and harvesting process compared to the overall mass of harvested duck cells. Higher moisture levels can result in a slight dilution effect, as protein content is typically measured as a percentage of the sample's dry weight. Introducing additional water increases the total sample weight without affecting the protein content, thereby diluting the concentration of proteins relative to the overall sample weight and resulting in a lower measured percentage of protein. The average moisture content across all five batches is considered acceptable as it is in line with the proposed specification (>80%). Low levels of fat (0.7 to 1.8 g/100g; mean 1.36 g/100g), carbohydrates (< 0.1 g/100g; mean < 0.1 g/100g) and ash (0.92 to 1.75 g/100g; mean 1.41 g/100g) were also identified.

Table 11. Overview of the proximate analysis of five batches of cell cultured duck (Confidential Appendix 8)

Compositional parameters	RB-001C	RB-001D	RB-001G	RB-002B	RB-002D	Mean	SD
Energy value (g/100 g)	66	67	69	45	40	57.4	13.80
Moisture (g/100 g)	84.2	84.1	83.4	85.3[1]	87.7[1]	84.94	1.69
Total fats (g/100 g)	1.8	1.7	1.7	0.9	0.7	1.36	0.52
Total Carbohydrate (g/100 g)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-
Protein (g/100 g) on as is basis	12.5	12.8	13.3	11.46[1]	10.3[1]	12.07	1.20
Ash (g/100 g)	1.75	1.65	1.53	1.19	0.92	1.41	0.34
Mass balance	100	100	100	100	100	100	-

[1]Lab processing error resulted in multiple testing of the sample. The value presented is the average of all data points taken as reported in Confidential Appendix 8.
SD= Standard Deviation

The parameters in Table 12 were selected to give a comprehensive overview of the nutritional profile of the biomass. The mineral content arises from inherent components of the cells and from carry-over nutrients from the culture media. Following harvesting, the cell cultured duck is separated from the culture media by centrifugation and washed with saline solution under centrifugal force. The washing in this step reduces any carryover of the spent culture medium. The analytical data on five batches of cell cultured duck show little variation in the mineral profile. Mean levels of macro minerals calcium, iron, potassium, phosphorus and magnesium were 13.04 mg/kg, 134.85 mg/kg, 3,640 mg/kg, 2,928 mg/kg and 223 mg/kg, respectively. Fluoride and selenium were below the limit of detection in all batches tested. Manganese was detected above the limit of detection in one batch (RB-001C) and zinc was also detected (mean value 28.3 mg/kg). The vitamin profile of cell cultured duck across five batches shows little variation in the vitamin content. The B-vitamins (B2, B3, B5, B6, B8, B9, B12), were all present. Vitamins A, E and K were not detected. A higher content of vitamin D3 can be observed for batch RB-002D (1.33 µg/100 g). This result is taken from a batch which had a higher moisture content which may influence the solubility and uptake of fat-soluble vitamins such as vitamin D3 leading to its increased uptake and storage. Nonetheless this increased level is not considered to be a food safety concern. Vitamin C, copper, molybdenum, iodine and chromium were not analysed in the biomass as they are not used in the media. Moreover, theese components are either present

in trace amounts or absent in coventional duck breast (Australian Food Composition Database - Release 2.0), and as such, not expected to be present in the cell cultured duck. The levels in conventional duck breast for these vitamins and minerals are: vitamin C 0mg/100g, copper, 0.34 mg/100g, molybdenum 2.5ug/100g, iodine 0ug/100g and chromium is not listed, and the levels in cell cultured duck is not expected to be higher. A targeted range of vitamins and minerals was assessed based on relevance to human nutrition and expected levels in the final biomass. Vitamin B1 and choline chloride are both used in the food safe culture media in the production of the biomass although they were not measured in the final cell cultured duck. Nevertheless, both vitamin B1 and choline are approved for use in food. Choline salts are listed in Schedule 15 of the FSANZ Food Standards Code under Category 12 with food additive number 100. Their use is permitted at levels consistent with good manufacturing practice (GMP) as a salt or salt substitute. Thiamin (vitamin B1) is listed in Schedule 17 of Food Standards Code as a vitamin approved without maximum level in meat extracts. Both vitamin B1 and choline are added to the media in trace amounts, and even in a conservative scenario where 100% of the substances added to the medium were carried over to the biomass, it is expected that the levels of choline and vitamin B1 would be low or below the LOD/LOQ in line with the other vitamins and minerals tested in the biomass that were added to the medium, and if present in the biomass they would not be present at levels of nutritional concern. Moreover, the culture media has been optimised to provide the cells with the nutrients required for proliferation, meaning that the nutrients added to the media are expected to be used by the cells and are not added to fortify the biomass.

Table 12. Overview of the Nutrient Composition of five batches of cell cultured duck (Confidential Appendix 8)

Compositional parameters	RB-001C	RB-001D	RB-001G	RB-002B	RB-002D	Mean values	SD
Sodium (g/100 g)	0.123	0.113	0.094	0.11	0.099	0.108	0.012
Calcium (mg/kg)	14.5	13.4	13.5	12.2	11.6	13.04	1.146
Iron (mg/kg)	197	139	110	110[1]	93.4	134.85	45.517
Fluoride (mg/kg)	<1	<1	<1	NA	<1	<1	-
Selenium (mg/kg)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	-
Potassium (mg/kg)	3,370	3,020	3,960	3,940	3,910	3,640	425.03
Phosphorus (mg/kg)	3,090	2,680	3,250	2,770	2,850	2,928	235.84
Magnesium (mg/kg)	206	190	268	227	225	223	29.25
Manganese (mg/kg)	0.34	< 0.30	< 0.30	< 0.30	< 0.30	0.308	0.018
Zinc (mg/kg)	34.3	29.1	26.4	26.5	28	28.30	3.643
Vitamin A, retinol (µg/100 g)	<21	<21	<21	<21	<21	<21	-
Vitamin B2, riboflavin (mg/100 g)	0.200	0.149	0.13	0.169	0.215	0.173	0.035
Vitamin B3, niacin (mg/100 g)	30.1	38.0	41.5	35.6	49.5	38.940	7.215
Vitamin B5 (as calcium pantothenate) (mg/100 g)	0.507	0.757	0.955	1.19	0.110	0.704	0.416
Vitamin B6, pyridoxine (mg/100 g)	0.275	0.224	0.349	0.29	0.309	0.289	0.046
Vitamin B8, biotin (µg/100 g)	33.4	39.4	46.1	43.2	47.5	41.92	5.68
Vitamin B9, folate (µg/100 g)	121	67.8	77.7	107	87	92.10	21.69
Vitamin B12 (µg/100 g)	0.785	0.517	0.447	0.407	0.402	0.512	0.160
Vitamin D3 (µg/100 g)	< 0.25	< 0.25	< 0.25	< 0.25	1.33	0.466	0.483
Vitamin E (mg/100 g)	< 0.08	< 0.08	< 0.08	< 0.08	< 0.08	< 0.08	-

Table 12. Overview of the Nutrient Composition of five batches of cell cultured duck (Confidential Appendix 8)							
Compositional parameters	**RB-001C**	**RB-001D**	**RB-001G**	**RB-002B**	**RB-002D**	**Mean values**	**SD**
Vitamin K1 (µg/100 g)	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	-

[1]Lab processing error resulted in multiple testing of the sample. The value presented is the average of all data points taken.
SD= Standard Deviation NA = not analysed. Issue with laboratory. Sample shipment between two laboratories in same company was delayed in customs.

The amino acid profile of cell cultured duck is included in Table 13. The highest amounts of individual amino acids identified in cell cultured duck were glutamic acid (1.409 g/100g), aspartic acid (1.018 g/100g) and the essential amino acids lysine (0.906 g/100g) and leucine (0.879 g/100g). Consistent results were obtained across four of the five batches tested for any individual amino acid. The amino acid content in batch RB-002D was lower compared to the other batches. A higher moisture content was also detected in this batch and whilst the moisture content itself doesn't directly affect the amino acid profile (the composition of amino acids present); it can affect the quantification of amino acids. This means that while the relative proportions of amino acids in the sample may remain the same, their absolute concentrations could decrease due to the dilution effect of increased moisture. Nonetheless, the lower values observed are still in line as a relative percentage of the composition of the novel food as seen in Table 14.

Table 13. Amino Acid Profile from five batches of cell cultured duck (Confidential Appendix 8)							
Amino acid	**RB-001D (g/100 g)**	**RB-001C (g/100 g)**	**RB-001G (g/100 g)**	**RB-002B (g/100 g)**	**RB-002D (g/100 g)**	**Mean values**	**SD**
Glycine	0.622	0.580	0.567	0.543	0.345	0.531	0.108
Histidine	0.296	0.286	0.273	0.26	0.166	0.256	0.052
Aspartic acid	1.15	1.08	1.10	1.1	0.658	1.018	0.203
Glutamic acid	1.64	1.53	1.51	1.45	0.915	1.409	0.285
Proline	0.540	0.526	0.499	0.505	0.297	0.473	0.100
Hydroxyproline	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	-	-
Serine	0.561	0.541	0.512	0.518	0.309	0.488	0.102
Ornithine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	-
Phenylalanine	0.531	0.511	0.488	0.461	0.296	0.457	0.094
Lysine	1.04	0.982	0.949	0.943	0.618	0.906	0.166
Methionine	0.262	0.280	0.273	0.241	0.144	0.240	0.056
Isoleucine	0.554	0.524	0.502	0.483	0.301	0.473	0.100
Leucine	1.03	0.985	0.935	0.883	0.563	0.879	0.185
Valine	0.677	0.638	0.614	0.584	0.367	0.576	0.122
Alanine	0.688	0.650	0.661	0.611	0.386	0.599	0.122
Threonine	0.546	0.519	0.503	0.491	0.295	0.471	0.100

Table 13. Amino Acid Profile from five batches of cell cultured duck (Confidential Appendix 8)							
Amino acid	**RB-001D (g/100 g)**	**RB-001C (g/100 g)**	**RB-001G (g/100 g)**	**RB-002B (g/100 g)**	**RB-002D (g/100 g)**	**Mean values**	**SD**
Tyrosine	0.446	0.443	0.415	0.392	0.259	0.391	0.077
Arginine	0.876	0.846	0.816	0.786	0.490	0.763	0.156
Cystine+cysteine	0.152	0.156	0.152	0.241	0.0940	0.159	0.053
Tryptophan	0.154	0.152	0.148	0.169	0.0955	0.144	0.028
*SD= Standard Deviation. Amino acids in bold text represent the essential amino acids.							

Table 14. Amino Acid profile in relative proportions from five batches of cell cultured duck							
Amino acid	**RB-001D (%)**	**RB-001C (%)**	**RB-001G (%)**	**RB-002B (%)**	**RB-002D (%)**	**Mean values**	**SD**
Glycine	5.66	5.17	5.19	5.09	5.23	5.27	0.23
Histidine	2.69	2.55	2.50	2.44	2.52	2.54	0.10
Aspartic acid	10.47	9.62	10.08	10.32	9.98	10.09	0.33
Glutamic acid	14.93	13.63	13.83	13.60	13.87	13.97	0.55
Proline	4.91	4.68	4.57	4.74	4.50	4.68	0.16
Hydroxyproline	-	-	-	-	-	-	-
Serine	5.11	4.82	4.69	4.86	4.69	4.83	0.17
Ornithine	-	-	-	-	-	-	-
Phenylalanine	4.83	4.55	4.47	4.32	4.49	4.53	0.19
Lysine	2.38	8.75	8.69	8.85	9.37	7.61	2.93
Methionine	2.38	2.49	2.50	2.26	2.18	2.36	0.14
Isoleucine	5.04	4.67	4.60	4.53	4.56	4.68	0.21
Leucine	9.37	8.77	8.56	8.28	8.54	8.71	0.41
Valine	6.16	5.68	5.62	5.48	5.56	5.70	0.27
Alanine	6.26	5.79	6.05	5.73	5.85	5.94	0.22
Threonine	4.97	4.62	4.61	4.61	4.47	4.66	0.19
Tyrosine	4.06	3.95	3.80	3.68	3.93	3.88	0.15
Arginine	7.97	7.53	7.47	7.37	7.43	7.56	0.24
Cystine+cysteine	1.38	1.39	1.39	2.26	1.37	1.56	0.39
Tryptophan	1.40	1.35	1.36	1.59	1.45	1.43	0.10
SD= Standard Deviation. Amino acids in bold text represent the essential amino acids.							

Cell cultured duck has a very low total fat content (1.36 g/100g on average). The fatty acid profile can be found in Table 15 below. It can be seen from the data below that there is low variability across the individual fatty acids in cell cultured duck.

Table 15. Fatty Acid Profile from five batches of cell cultured duck (Confidential Appendix 8)							
Fatty acid	**RB-00C1 (% relative)**	**RB-001-D (% relative)**	**RB-001-G (% relative)**	**RB-002-B (% relative)**	**RB-002-D (% relative)**	**Mean values**	**SD**
Omega 3 fatty acids	1.08	1.15	1.14	< 0.05	< 0.05	0.69	0.59
Omega 6 fatty acids	0.32	0.36	0.09	0.24	< 0.05	0.17	0.12
C4:0 butyric acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C6:0 Caproic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C7:0 Enanthic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C8:0 Caprylic acid	<0.05	<0.05	<0.05	0.15	<0.05	0.05	0.00
C9:0 Pelargonic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C10:0 Capric acid	0.09	0.10	0.12	0.09	<0.05	0.09	0.03
C11:0 Undecyclic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C11:1 Undecylenic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C12:0 Lauric acid	0.23	0.25	0.26	0.29	0.13	0.21	0.05
C12:1 Lauroleic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C13:0 Tridecyclic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C13:1 Trydecylenic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C14:0 Myristic acid	1.89	2.05	1.70	1.55	1.63	1.79	0.18
C14:1 (n-5c) Myristoleic acid	0.14	0.18	0.17	0.13	0.16	0.15	0.02
C14:1 (n-5t) Myristoleic acid	0.13	<0.05	<0.05	<0.05	<0.05	0.08	0.04
C15:0 Pentadecyclic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C15:1 (n-5c) Pentadecenoic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C15:1 (n-5t) Pentadecanoic acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.00
C16:0 Palmitic acid	22.75	22.68	24.16	23.39	25.35	24.24	1.56
C16:1 (n-7c) Palmitoleic acid	4.80	5.25	4.97	5.30	5.66	5.06	0.41
C17:0 Margaric ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C17:1 (n-7c) Heptadecenoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C17:1 (n-7t) Heptadecenoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–

Table 15. Fatty Acid Profile from five batches of cell cultured duck (Confidential Appendix 8)							
Fatty acid	**RB-00C1 (% relative)**	**RB-001-D (% relative)**	**RB-001-G (% relative)**	**RB-002-B (% relative)**	**RB-002-D (% relative)**	**Mean values**	**SD**
C18:0 Stearic ac.	15.28	14.86	14.74	14.12	14.71	14.74	–
C18:1 (n-6c)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:1 (n-7c) Vaccenic ac.	4.10	4.23	4.00	4.42	4.57	4.26	–
C18:1 (n-7c) trans Vaccenic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:1 (n-9c) Oleic ac.	43.71	43.28	43.12	45.22	42.27	43.52	–
C18:1 (n-9t) + C18:1 (n-12t)	1.67	1.71	1.87	1.73	1.86	1.77	–
C18:2 (9c, 11t) Conjugated linoleic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:2 (n-6c) linoleic ac. (LA) ω6	0.32	0.36	0.09	0.24	<0.05	0.21	–
C18:2 (n-6c) linoleic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:2 t2	<0.05	0.13	0.13	<0.05	0.10	0.09	–
C18:3 (n-3) α-linoleic ac. (ALA) ω6	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:3 (n-6) γ-linoleic ac. (GLA) ω6	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:3 t3 (C18:3 t1+C18:3 t2)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C18:4 (n-3) Moroctic Ac. ω3	1.08	1.15	1.03	<0.05	<0.05	0.67	–
C19:0 Nonadecylic ac.	0.26	0.3	0.26	0.27	0.3	0.28	–
C19:1 (n-12t)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C19:1 (n-9t)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:0 Arachide ac.	0.38	0.38	0.33	0.29	0.33	0.34	–
C20:1 (n-9c) Gondoic ac.	1.83	1.68	1.69	1.7	1.67	1.71	–
C20:1 (n-9t) + C18:2 (10t, 12c)+C20:1 (n-15c)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:2 (n-6c) Eicosodienoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:3 (n-3c) Eicosatrienoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:3 (n-3c) Eicosatrienoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:4 (n-6c) Arachidonic ac. (AA) ω6	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C20:5 (n-3c) Eicosapentaenoic (EPA) ω3	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C21:0 Heneicosanoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:0 Behenic ac.	0.23	0.27	0.19	0.19	0.22	0.22	–
C22:1 (n-11) cetoleic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:1 (n-9c) Erucic ac.	0.44	0.44	0.34	0.35	0.39	0.39	–
C22:1 (n-9t) Brassidic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:2 (n-6c) Docosadienoic ac.	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:3 (n-3c) + C22:4 (n-6c)	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:5 (n-3c) Docosapentaenoic ac. (DPA) ω3	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:5 (n-6c) Docosapentaenoic ac. ω6	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	–
C22:6 (n-6c) Docosapentaenoic ac. ω6	<0.05	<0.05	0.10	<0.05	<0.05	0.05	–
C24:0 Lignoceric ac.	0.34	0.37	0.43	0.32	0.36	0.36	–

Table 15. Fatty Acid Profile from five batches of cell cultured duck (Confidential Appendix 8)							
Fatty acid	**RB-00C1 (% relative)**	**RB-001-D (% relative)**	**RB-001-G (% relative)**	**RB-002-B (% relative)**	**RB-002-D (% relative)**	**Mean values**	**SD**
C24:1 Nervonic ac.	0.33	0.34	0.3	0.26	0.27	0.30	-
SD= Standard Deviation-							

B.6.3. Stability

B.6.3.1 Stability of cell cultured duck

The stability of cell cultured duck was determined by assessing the physical, chemical and microbiological parameters in three batches each tested at three different time points which are representative of the storage conditions of the novel food before its use. The selected parameters were tested in each batch (RB-002B, RB-002C and RB-002D) according to the following timepoints in order to test the specifications of the novel food:

- Timepoint 0: Thawed and refrigerated sample ready to be tested
- Timepoint 1 (T_{24hr}): 24 hours post-thawing, refrigerated (4-5°C)
- Timepoint 2 (T_{48hr}): 48 hours post-thawing, refrigerated (4-5°C)

The report for the stability testing can be found in Confidential Appendix 13. A summary of the results can be found below.

B.6.3.1.1 Microbiological assessment

Review of the total microbiological profile for all lots over the course of the 48-hour study is determined as acceptable for the intended use. There are no observable concerns related to safety or quality. No microbial growth of significance was observed in all tested microbial indicators and pathogens except for Total Aerobic Count (TAC).

Of note from the reported data, the TAC data for lot RB-002-B at T_{0hr} and T_{48hr} RB-002-C were above the LOD and LOQ. As indicated in B.6.2., due to lab sampling processing error, multiple samples were tested at T_{0hr} for RB-002-B with a total of four samples tested. The average TAC of these samples was above the LOQ however, all values were within the proposed specification for TAC for cell cultured duck (< 3,000 (cfu/g)).

To verify that the observed TAC counts were attributed to the non-sterile operations of the process and not representative of the sterile operations, a pre-harvest retention sample collected from the bio was submitted to an external laboratory to verify the sterility of the bioprocess for lot RB-002-B. The result of this test was negative for microbial contamination (Confidential Appendix 14). The TAC results recorded for this sample at time points of T_{24hr} and T_{48hr} demonstrate that there was not a significant increase in TAC counts from the T_{0hr} results.

For batch RB-002-C, the average TAC for both T_{24hr} and T_{24hr} was reported as being below the LOD. An increase in TAC is observed at T_{48hr} above LOD. In isolation, this result can be interpreted as a $10^{\wedge 2}$ increase in TAC counts for that lot, but if all lots are taken into context, this result can also be interpreted as a minor increase with consideration to the variance observed in RB-002-B, T_{0hr}.

Lot RB-002-D showed no significant growth.

The variance in microbial counts at T_{0hr} can be attributed to a number of factors which include non-homogenous distribution of aerobic plate count (APC) in the product matrix due to the washing and harvesting method, the use of non-sterile food packaging bags, and or mild contamination during sample collection or handling by operators. Gourmey's products adhere to a positive release protocol meaning they would not be

released to market until confirmed in specification. In the event of out-of-specification results at time of production, they would not be released, and corrective actions would be taken to investigate and appropriately assign a disposition for the product.

B.6.3.1.2. Proximate and compositional assessment

The protein, moisture and iron content remained consistent across the batches over the time period tested. No significant degradation was observed for moisture, protein, and iron.

A lower protein value and higher moisture content is exhibited at T_{0hr} for batch RB-002-C. This can be attributed to residual moisture retained in the sample from the washing and harvesting process compared to the overall mass of harvested duck cells. Following centrifugation, sufficient settling time can lead to a gradient where residual moisture separates from the cells. Depending on when and where the sample was collected from the centrifuge bowl, there may be slight variations in the moisture content. Higher moisture levels can result in a slight dilution effect, as protein content is typically measured as a percentage of the sample's dry weight. Introducing additional water increases the total sample weight without affecting the protein content, thereby diluting the concentration of proteins relative to the overall sample weight and resulting in a lower measured percentage of protein.

B.6.3.1.3. Media residues assessment

B.6.3.1.3.1. Poloxamer

Poloxamer levels remained consistent over the 48-hour period in batch RB-002-B. An increase was observed in both RB-002-C and RB-002-D. Mechanical disruption from harvesting procedures (centrifugation and packaging) and freeze-thaw cycles can cause physical disruption of cell membranes resulting in the gradual leakage and release of intracellular contents. Poloxamers are amphiphilic compounds, meaning they have both hydrophilic and hydrophobic segments. This dual nature enables versatile interactions with cell membranes and a range of biological and chemical substances. These interactions stabilise interfaces and reduce surface tension which can enhance the solubility and stability of various compounds. In cell culture, poloxamers align at liquid interfaces to form protective barriers around cells, significantly mitigating mechanical stress, shear forces, and reducing cell adhesion. It is observed that poloxamers have membrane sealing properties by interacting with lipid monolayers and can be incorporated into the phospholipid bilayer of cell membranes (Wu et al., 2005). This incorporation helps stabilise and protect cell membranes from damage (Kerleta et al., 2010). Ruptured cell membranes during harvest and freeze-thaw cycles can, over time, explain a gradual increase in the total free poloxamer that can be detected in the duck cell biomass. Nonetheless, the levels of poloxamer observed are considered safe as per Section C.2.1.5.1.4.

B.6.3.1.3.2. Ethanolamine

The results of ethanolamine remain within the proposed specification of < 30 µg/g for duck cell culture biomass and are therefore acceptable. A percent relative standard deviation of (%RSD) of 20% is accepted by the laboratory where this analysis was carried out. Although a higher %RSD indicates greater variability in the measurements, the variation on the different timepoints is larger than 20% so there is a noted upward slope in the ethanolamine through 48 hours.

As above, an increase in ethanolamine levels after the duck cells have been harvested from culturing could be due to the mechanical disruption of harvesting procedures leading to the disruption of cells membranes which could lead to a gradual increase the total free ethanolamine detected in the duck cell biomass. Disruption of membranes during harvesting can lead to the breakdown of phosphatidylethanolamine (PE) which is a major component of cell membranes. Ethanolamine is a naturally occurring organic compound which is essential for the formation of cell membranes as a precursor in the biosynthesis of PE. The more extensive the membrane damage, the greater the release of ethanolamine and other intracellular metabolites. This is supported by findings in various studies on PE metabolism and membrane integrity (Lipton et al., 1990, Shin and Moore, 1990).

Enzymatic activities post-harvest may also contribute to the release of ethanolamine from phospholipids. Phospholipase D-mediated hydrolysis of PE leads to the production of ethanolamine which can be stimulated by factors such as scraping during cell collection and exposure to certain chemicals like phorbol ester (Kiss and Anderson, 1989).

Enzymatic activities often persist beyond initial processing in conventional meat and plant-based food products which can contribute to the degradation of cellular components throughout the shelf-life of a product. Hence, the findings in the data are in line with the expectations for the presence of ethanolamine in foods.

B.6.3.1.3.3. Insulin

Recombinant bovine insulin was below the LOD for each lot and time point measured.

B.6.3.1.4. Conclusions on stability

Overall, based on the data above, it can be concluded that cell cultured duck maintain stability when refrigerated (4-5°C) for up to 48 hours. The microbiological and compositional analyses consistently showed stable results throughout the study period, supporting their suitability as a food ingredient for further processing into food products meeting the specifications for cell cultured duck as a novel food ingredient.

Since the product will be stored frozen, microbiological changes will not occur during the storage period (Villegas-Cayllahua et al. (2024)). Shelf-life will be then limited by physico-chemical reactions and undesired changes in the properties of the ingredient. Lipid oxidation is one of the main causes for quality loss during the storage of frozen meat as reported by Villegas-Cayllahua et al. (2024). Ice crystals formed during freezing may break the cell membrane, hence releasing compounds such as enzymes to the extracellular space. Enzymes and other chemicals can catalyse these reactions, although the speed rate of these changes will depend on numerous factors including temperature. Peroxides are primary metabolites produced as a result of these chemical reactions. These compounds are unstable and will typically quickly react with other substances in the matrix to form aldehydes and other chemicals such as malonaldehyde, which commonly used as an indicator of these processes. The presence of these substances in food induces a rancid, bitter taste, as well as changes in colour. There are not legal limits established for the presence of these compounds in food. Along with (Villegas-Cayllahua et al., 2024) some studies demonstrated that chicken and goose meat can be stored for 6-12 months under freezing conditions, although negative effects on certain nutrients occurred period (Tomás, 1990, Wereńska and Okruszek, 2022).

As mentioned, lipid concentration and profile, temperature and other factors will heavily influence the shelf-life of these products under the proposed storage conditions. Based on the low concentration of fat in the product, lipid oxidation is not expected to be significant in the biomass. In products such as vegetables (which are typically low in fat), stability under freezing conditions is caused by other events such as water sublimation and breakdown of nutrients such as vitamin C. It is important to note that freezing also affects the physical structure of the food, hence denaturalizing proteins and causing undesirable changes in their texture and appearance.

Taking this together, lipid oxidation is unlikely to occur based on the low concentration of fats and reducing sugars. Considering this, it is reasonable to assume that the product will be stable when frozen for at least one year.

B.6.3.2. Stability of Finished Products

The cell biomass will be used to manufacture duck products such as pâtés and foie gras. These products are typically subject to minimal processing and heat-treatment. The cell biomass is not expected to have any detrimental impact on the shelf-life of these products. Acrylamide and other compounds are unlikely to be produced during the cooking as a result of the inclusion of cell cultured duck. The pâtés and foie gras produced using cell cultured duck would not subjected to the typical processing of high-temperature cooking associated with high temperature cooking of starchy food products (frying, baking, roasting and also industrial processing at +120°C and low moisture) (EFSA, 2015). Most of the final products will be frozen, hence no

changes are expected to occur during storage. Consequently, stability in food matrices is deemed not necessary for cell cultured duck.

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

Cells as food ingredients cannot be quantified via conventional methods. Instead, we propose an analytical method for the verification of the declared species. For more information, please see Section B.4.2.2.1.

C. Safety Assessment

The information and data to support the safety of Gourmey's cell cultured duck has been addressed 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 (FSANZ, 2024a). The components used to establish the overall safety of cell cultured duck have been addressed in the following sections taking into account the information requirements listed in the guidelines.

Guideline 3.5.2. (Novel Foods)

- C.6 Food Ingredients derived from a new source
    - *C.6.1 Information on the safety of the source organism (Section B.4, C.1)*
    - *C.6.2 Information on the composition of the novel food (Section B.5, B.6)*
    - *C.6.3 Information on the toxicity of the novel food ingredient derived from the new source (Section C.1-C.5)*
    - *C.6.4 Safety assessment reports prepared by international agencies or other national government agencies (Section C.6)*
- C.7 Foods produced by a process not previously applied to food
    - *C.7.1. Details of the process not previously applied to food (Section B.4)*
    - *C.7.2 Information on the toxicity of the novel food produced by a process not previously applied to food (Section C.1-C.5)*
    - *C.7.3 Safety assessment reports of prepared by international agencies or other national government agencies (Section C.6)*

C.1. History of safe consumption

C.1.1. History of use of the source

The cells used in production process of Gourmey's cell cultured duck were isolated from fertilised duck eggs. Duck meat and eggs have been traditionally consumed in the Australia and New Zealand and other regions. Fertilised eggs are commonly consumed in some Asian countries including the Philippines, Thailand, Cambodia, Laos and China.

Preparations such as foie gras, pekin duck and others are served and consumed in Australia and New Zealand. Ducks are not known to produce and/or store any toxins in their tissues.

C.1.2. History of use of cell cultured duck

Whilst there is no history of consumption of cell cultured duck, duck meat is widely consumed globally. Moreover, the nutritional value of the cell cultured duck is adequate and safe as reported in Section E The biological and chemical safety has been extensively characterised in the dossier and no concerns have been identified, hence the novel production process does not give rise to food safety concerns and the duck cells are considered to be safe.

Although cell cultured duck has not yet been commercialised in any region globally, cultured chicken cells (fibroblasts) were first authorised in Singapore in 2021. A few establishments have been serving cultured chicken preparations such as cultured chicken breast and nuggets. A study published by Chong et al. (2024) assessed the sensory characteristic of cultivated chicken consumed by 107 diners in Singapore. Whilst this

study was not aimed at looking at safety, it is one of few published studies to document the consumption of cultivated chicken. No adverse effects on human health have been reported in Singapore.

More recently cell cultured quail was approved as a novel food in Singapore (April 2024) and is being served in a number of restaurants in the region.

Additionally, cultured chicken cells (fibroblasts and myoblasts) have recently been evaluated by the US authorities (FDA and USDA) and deemed safe for human consumption. Preparations with these ingredients have been served in the US on several occasions. No adverse effects derived from their consumption have been reported in the US.

Cultivated quail was the first cultivated meat product accepted by FSANZ. FSANZ have completed their scientific risk assessment, and concluded that cultured quail is non-pathogenic, non-toxic, and is safe for use as an ingredient in food, under the intended conditions of use and at the estimated dietary intake levels. The application moved to the public consultation phase, which was closed in February 2024. Following public feedback and potential amendments to the Australia New Zealand Food Standards Code, the final approval for cultured quail is anticipated by mid-2025.

In January 2024, cultured beef received a “no questions” letter from Israel’s Ministry of Health (MoH), granting pre-approval to market this product in Israel. The approval is the first-ever green light for a non-chicken cultivated meat product.

The risk of consuming cell cultured duck is detailed below.

One of the major concerns discussed in the literature is the tumorigenic potential of the cells. It should be noted that no viable cells can be reasonably expected to be present in cell cultured duck. The tumorigenicity of the cells has not been assessed analytically since the risk of ingested cells leading to tumour formation is considered to be extremely low. For cell cultured duck to form a tumour, these would firstly need to possess tumorigenic potential, then survive food processing and cooking, resist chemical digestion, cross the gastrointestinal barrier intact, enter into blood circulation, evade the immune system and finally proliferate in the human body (FAO, 2023). Moreover, as the cells belong to avian species, successful colonization of human tissue would be extremely rare.

The oral consumption of ESCs has been studied for therapeutic use (Liu et al., 2023), but the oral route presents various limitations. For example, ESCs need to be kept under strict conditions in order to preserve their viability, which is extremely hard to achieve in oral delivery systems. ESCs are generally kept below -80 ºC to preserve their viability. Besides this technical limitation, the acidity of the gastric chyme, bile salts and digestive enzymes still pose a significant hurdle (Luo et al., 2022, Liu et al., 2023). Additionally, even in the remote event where cells could successfully reach the gastrointestinal tract intact, the epithelium and mucus layer act as physical barriers. The gastrointestinal flora may also produce and secrete enzymes that may contribute to digesting and breaking down any living material that has made it to the gastrointestinal tract (Liu et al., 2023). Moreover, cells that are used in food are further processed (freezing, processing, cooking), which would further limit their ability to survive.

Based on the risk assessment conducted, the tumorigenic potential of cell cultured duck is not a food safety concern because:

- The cell cultured duck is immediately frozen after harvest. When cells are frozen without a cryoprotectant, ice crystals form rupturing the cell membrane leading cell death (Dumont et al., 2006).
- Subsequent processing and cooking steps would serve as additional kill steps, meaning the likelihood of consuming viable cells is extremely low. The processing and storage conditions to which the cell cultured duck is subject are designed to maintain its physical-chemical properties but not to maintain cell viability post-harvest.
- Further, in the unlikely event that viable cells remain after freezing, processing and cooking, they would not be able to survive being ingested due to chemical digestion, where stomach acid and digestive enzymes catabolize the cells into smaller components that are either absorbed or excreted (Patricia and Dhamoon, 2023).

- The stability of the cell line is closely monitored. Any significant changes in the growth characteristics, morphology, pluripotency and the ability to form EB would be the first indicators of undesired phenotypic and genotypic changes in the cell culture.
- Finally, the probability of all these events occurring concurrently is such that it was not possible to identify a credible pathway to harm (FAO, 2023).

C.2. Assessment of Cell Culture Medium Components

Gourmey has implemented supply-chain preventative controls and a supplier management program in line with the Food Standards Code, hence ensuring the quality and safety of the raw materials and ingredients used in the process of making cell cultured duck. Since the cell cultured food industry is still in its infancy, many of the materials used for cell culturing are commonly used in the manufacturing of pharmaceuticals, therapeutics, and or medical devices that can be sourced from reputable suppliers in the context of those industries. Because these suppliers would not have been expected to have prior knowledge of food regulations or experience with supplying materials or ingredients for use in food products, each component has been evaluated and risk assessed to ensure that potential hazards and risks have been assessed and prevented from being introduced during the production of cell cultured duck. Gourmey has prioritized sourcing materials from reputable suppliers in the food, pharmaceutical, and therapeutics industries.

Before any ingredients are incorporated into the formula, an internal assessment is carried out to ensure their safety and adequacy. These two conditions must be met:

- Have a long-standing history of safe consumption or use in New Zealand, Australia and/or other regions or have solid toxicological data supporting their safety for the consumer.
- Be compliant with our internal specifications and quality standards (e.g., microbiological quality, residues, identity, etc.).

Different culture medium formulations and components are used at different stages of production. For the early stages of isolation, components and reagents were used that do not have a history of use in food and were Research & Development (R&D) or pharma grade. These components are essential for biological processes and cell requirements during the sensitive stage of isolation. Once the cells were isolated, they were adapted over time to a chemically defined food-safe medium. Each time the cells were passaged, the medium was changed, thus diluting the original components. Hence, it is not reasonably likely that any components used in the early isolation stages would be present in the final biomass product due to an exponential dilution effect and expansion of the cells over a significant period of time.

Gourmey’s cell cultured duck is derived from duck embryonic stem cells (dESC). Embryonic stem cells (ESCs) exhibit many intrinsic properties that enable them to proliferate extensively in culture compared to somatic cells such as fibroblasts. These properties include their pluripotency, high telomerase activity, and accelerated cell cycle that enables rapid proliferation. The accelerated cell cycle of ESCs facilitates rapid proliferation, maintaining the cells' undifferentiated state across numerous passages. The inherent pluripotency of ESCs, coupled with their high telomerase activity, ensures that they can replicate without succumbing to the telomere shortening that typically limits cellular lifespan (Hayflick limit). This characteristic, combined with their genetic stability, contributes to the longevity of ESC cultures, enabling them to undergo many more passages than cells such as fibroblasts. Because ESCs can undergo more passages than primary cell lines, it translates into a higher dilution factor for any accumulated substances within the cell culture over the production cycle. Each passage involves splitting the cells and introducing them to fresh media, effectively diluting metabolic byproducts, extraneous compounds from media, and cellular waste. This process repeated more frequently due to the number of passages ESCs are subjected to.

Therefore, it is highly unlikely that any components used in the early isolation stages would be present in the final biomass product due the expansion and dilution of the cells over a significant period of time. Consequently, the applicant has only tested the final biomass for the presence of residual media components in the culture media used from MCB to harvest.

A risk assessment of each component has been performed and for media components used in the early stages of isolation and adaptation that pose a potential risk would have been diluted to below the threshold

of toxicological concern (if a limit exists) in the subsequent passages taken to create the cell banks and any media components remaining in cell cultured duck and finished products will be below the limit of detection (LOD).

Culture medium formulations and components that the cells have been exposed to are provided in Confidential Appendix 5a - Media Composition & Risk Assessment and Confidential Appendix 5b – Extensive Media Component Risk Assessment. The certificates of analysis for the animal-derived media components and recombinant proteins used in the early stages of isolation are provided in Confidential Appendix 5ci and 5cii.

Gourmey's MCB and WCB were created using a chemically defined food-safe medium. For the growth and expansion of the cell cultured duck, a chemically defined food-safe cell-culture medium is used. The cell culture medium consists of common compounds found in animal feed and human food including amino acids, fatty acids, sugars, trace elements, salts, and vitamins. Most culture media components are similar to those employed in standard fermentation processes for the production of food enzymes and other fermentation-derived ingredients, which have a long history of safe use in food production.

The primary source for potential chemical hazards introduced in the process are the cell culture medium components, food contact materials, food contact materials used in the process, toxicity, and the allergenicity of the cells. The risk assessment for each potential source is discussed.

The potential for radiological hazards has also been assessed and a detailed risk assessment is included in the Food Safety Plan (please refer to Confidential Appendix 3). Gourmey does not use ionizing radiation, such as gamma irradiation on its products. Additionally, the process location, as described in the Food Safety Plan, is situated more than 80 kilometres from any known nuclear power plants. This distance places the production facilities outside the standard radius of an Ingestion Pathway Zone (IPZ). The IPZ is a defined area approximately 80 kilometres around a nuclear power plant, where radiation exposure through ingestion of contaminated water or food might be possible.

Culture media components used to culture the duck cells to be used as a food ingredient are food-safe and, where applicable, meet the relevant quality specification standards such as the Food Chemicals Codex (FCC).

C.2.1. Biological Risks

Biological risks derived from bacteria and fungi are covered under the Food Safety Plan (please refer to Confidential Appendix 3). These are common and non-emerging risks in the food industry and, consequently, do not require regulatory consideration. All these risks are prevented and controlled by standard practices such as the application of cGMP in media handling and manufacture and a correct supplier control. The risk of prions, although not unique to the cultured meat industry, is discussed below.

For bacteria and fungi, standard laboratory practices are followed to prevent the contamination of the cells: use of UV light to disinfect cabinets, disinfection of surfaces with isopropanol at 70%, filtration of the media, sterilized consumables, etc. Virucidal and sporicidal agents are frequently used to clean surfaces and eliminate any potential viruses and bacterial spores.

Environmental monitoring has been implemented to ensure the effectiveness of the cleaning and disinfection procedures.

C.2.1.1. Foetal Bovine Serum

Foetal Bovine Serum (FBS) was used during the early stages of isolation and adaptation. It is not used in the food-safe culture medium used to produce cell cultured duck.

FBS is obtained from the blood of bovine foetuses during slaughter. This ingredient has been extensively used for decades in the manufacture of biologics and other drugs due to its unique composition of growth factors and other essential nutrients that help sustain the proliferation and survival of cells *in vitro*. The main

risks to using FBS are high variability and potential contamination with bacteria, viruses or prions. Since FBS has been a vital ingredient in the manufacture of biologics, its use is strictly regulated and there are specific requirements for the use of FBS and other blood-derivatives. Currently there are no regulatory requirements for the use of FBS in food production. Since pharmaceuticals provide a more stringent regulatory framework, FBS complying with the conditions in EMEA (2003), guidelines adopted by the Therapeutic Goods Administration (TGA, 2014), provides a sufficient guarantee that the product is safe for use in food. The main risks related to the use of FBS in food is the potential presence of adventitious microorganisms and zoonotic viruses, mainly *Mycoplasma* spp., bacteria and fungi, and viruses (bovine pestiviruses, bovine adenovirus, bluetongue virus, bovine parainfluenza virus 3, rabies virus, bovine parvovirus, bovine herpesvirus 1, bovine respiratory syncytial virus, and reovirus). It should be noted that transmissible spongiform encephalopathies (TSE) such as bovine spongiform encephalopathy (BSE) are not a risk for blood and blood-derivatives (provided that certain slaughter and stunning methods have not been used) regardless of the BSE risk in the sourcing territory as stated by the World Organization for Animal Health (OIE) in chapter 11.4 (Article 11.4.1) (WOAE, 2021).

FBS was used in the steps prior to the MCB creation. The FBS used was tested in compliance with EMA guidance (EMEA, 2003) and was sourced from either Australia and/or New Zealand, which are low risk for TSEs. FBS is not used during the cell proliferation and expansion steps used to produce the cell cultured duck. It is therefore safe to conclude that no food safety risks are introduced by the use of FBS in cell cultured duck.

C.2.1.2. Prion Risk

The risk of infectious prions in cultured meat has been mainly associated with spontaneous production of prions during cell growth and the carry-over from blood derivatives. In fact, prion diseases have been reported to be transmitted through blood on a few isolated occasions (WHO, 2006, ECDC, 2021). However, the cases in the literature correspond to cases where patients have received blood transfusions or plasma-derived medicinal products produced from infected human donors. Yet, there are no known cases of humans being infected by consuming products made from animal blood. It should be noted that bovine blood and derivates are no longer considered risk products for the transmission of spongiform encephalopathy (BSE) as long as the animals have not been subject to stunning processes that increase the contamination with brain and nervous system particles (as previously mentioned). Most prion agents and diseases are known to affect mammalians such as the bovine, ovine and human species. The scientific evidence, however, indicates that poultry are inherently resistant to prions, although most research has been done in chickens. Scarce information is available on prionic diseases in ducks.

The lack of reported prion diseases in avian species may be explained by the apparent natural resistance to prion formation in avian, lack of general research in this area (perhaps correlated to the lack of remarkable cases of “unknown” diseases in domestic and wild avians) and low tolerance towards avian diseases in livestock farming. Animals showing any pathologies, diseases and/or slow development are typically euthanized, which is in many cases a compulsory practice under the current animal health regulations. It is noteworthy that these practices are more common in poultry and other avian as these animal species exhibit quicker growth and take significantly less resources than large production animals such as pigs, cows or steers.

As regards to the risk of prions being spontaneously produced during cell growth, the mechanisms by which the prion protein (PrP^{c}) is spontaneously and abnormally folded into its pathological counterpart (PrP^{Sc}) are still not clearly understood. Different *in vitro* models have been used to identify key elements in the formation of prions in the cell, being mammalian and yeast models the most prominent examples in the prion research space. According to the review performed by Wisniewski (Wisniewski et al. (2018), the occurrence of prions in yeast is approximately less than one in a million cells per generation. However, this may not be accurately representative of mammalian or avian cells. The mechanism behind the formation of prions have been predominantly studied in transgenic mice with an overexpression of the PrP^{c} in their brains and, therefore, genetic predisposition to the formation of amyloid aggregates.

Studies indicate that spontaneous prion formation is rare, but the formation rate is not reported. PrPc is a natural cellular component that plays a fundamental and (yet) not entirely clear role in animals and humans.

In fact, PrP^c has been linked to the regulation of the pluripotency, self-renewal and differentiation capacities in embryonic stem cells (Lopes and Santos, 2012, Miranda et al., 2011, Peralta et al., 2011), although the research is mostly on mouse cells.

A recent publication (Jeong et al., 2021) was the first to report the sequence of the prion protein (PRNP) gene in Pekin ducks and pointed some key differences in between chicken and duck PRNP sequences which the study claimed that may influence susceptibility to prions in ducks. The study showed that the Pekin duck PRNP sequence is more similar to that of geese (97.3%) than chickens (82.7%) or mallards (wild ducks) (82.4%). The higher proportion of β-sheets in Pekin duck PRNR may increase susceptibility to prion diseases as these are predominant structural conformations in PrP^{Sc} (pathogenic form of the prion protein pr PrP^c). This was linked to the higher aggregation propensity found in Pekin duck PRNP in computational analyses. However, limitations exist due to the exclusive use of *in silico* analyses rather than *in vitro* or *in vivo* studies, low number of individuals used (4 ducks) and lack of representativity of the different Pekin duck breeds. Moreover, despite the tentative findings, no prion diseases have been so far documented in ducks. Other studies have indicated that duck PRNP sequence shows high similarity with that of chickens (97%) and very low similarity with mammalian PRNP (< 30%) (Wang et al., 2004). The low similarity exhibited between avian and mammalian PRNP sequences may be an indicator of the likelihood of a cross-species leap, which should be presumed to be low. Further to this, Dima and Thirumalai's findings (2002) suggest that avian prion PrP^c are less propense to fold into β structures due to the helical regions identified in it. However, as previously mentioned, no duck prion diseases have been identified at the moment. Prion formation is especially relevant in neurons and other cells of the nervous system.

There are studies suggesting that mesenchymal murine stem cells can be infected by prions in neurogenic differentiation conditions (García-Mendívil et al., 2021). This could respond to the neuron-like features that the cell was displaying. However, the authors concluded that stem cells in growth conditions (not in a neurogenic differentiation state) were not "permissive to prion infection".

Considering that our cells are undifferentiated, that these do not exhibit any neuron-like features and that prions in avian have not been reported, it is safe to conclude that the formation of prions in the cell line is not a concern. Moreover, as detailed at the beginning of this section, serum-derived ingredients are not vectors of prions and, consequently, their use is not a risk.

C.2.1.3. Viruses

A risk assessment for each potential virus of concern for animal and human health has been performed. Relevant viruses listed in the Australian Government National list of notifiable animal diseases have been considered (DAFF, 2024a). It should be noted that international regulatory agencies have repeatedly concluded that these viruses cannot be transmitted through food. Moreover, cultured meat companies require animals (or their products) from which the cells are sourced to be healthy, free from any diseases and vaccinated against certain diseases as per the applicable legislation. This limits the risk of any viruses being introduced into the cell line and the final product. Furthermore, lytic activity would most likely be observed during cell culturing, leading to a decrease in viability and ultimately the death of the culture. The assessment demonstrates that viruses would not be considered a food safety concern nor an emerging risk when cultivating animal cells for human consumption. Moreover, as outlined in Section B.4.2.3., a non-targeted transcriptomic analysis using NGS (Next Generation Sequencing) was performed to detect the potential presence of replicative viruses, along with species-specific PCR tests, all of which demonstrated that the cells were free from replicative viruses and adventitious agents (Confidential Appendix 6a).

Viruses from avian species

Avian influenza

Influenza viruses are single-stranded, negative-RNA strand viruses belonging to the *Orthomyxoviridae* family. These are classified into A, B and C types based on their antigenic features (Sendor et al., 2023). Influenza type A viruses mainly affect domestic and wild birds but can occasionally infect humans and other animal species. The migration of infected wild birds plays a key role in the transmission of the virus across multiple geographical areas. Transmission to humans usually occurs via direct contact with live animals, carcasses,

or raw meat. However, the virus has low replicative capacity in humans, hence making human-to-human transmission a rare and isolated event (Capua and Alexander, 2002). Current practices require all birds in an identified or suspected case of avian influenza to be culled to prevent the spread of the infection.

Over the last decades, only H5N1 and H7N9 strains (also known as Highly Pathogenic Avian Influenza or HPAI based on their mortality rate in birds) have been involved in the outbreaks in humans until the recently first reported cases of H5N8 (Kanaujia et al., 2022). Humans will commonly develop flu symptoms (muscle pain, fever, cough, migraines, etc.) that can, in some cases, lead to more severe and life-threatening complications (CDC, 2022).

As previously detailed, healthy and ill birds are required to be culled, and all infected materials (e.g., eggs) destroyed based on the current standard practices, hence limiting the spread of the diseases through animal-derived ingredients and carcasses. Additionally, although the disease can be asymptomatic, HPAI viruses quickly spread to other birds, with symptoms starting after a few days, and have very high mortality rate, which make detection easier than in LPAI (Low Pathogenicity Avian Influenza) types. Hence, it is unlikely for any contaminated meat or egg to end up in the food chain. Moreover, it is a notifiable animal disease, and any cases should be communicated to the competent authority in the region.

The risk of contracting the disease via the consumption of contaminated meat or eggs is also extremely low if cooked properly (DAFF, 2024b), although most safety agencies reiterate that there is no evidence that the virus can be contracted by consuming poultry products. However, the WHO (2018) reported a few cases of H5N1 that have been linked to the consumption of raw poultry blood but not to other poultry products. Considering the above, the overall food safety risk is extremely low compared to that of other biological agents and may even be non-existent. In the cell culture, lysis would be observed along with low viability numbers until the death of the culture (Samji, 2009).

The only avian-derived ingredient in the process are the sourced eggs. As mentioned in this document, eggs are obtained from trusted and authorized farms with no history of influenza A.

Newcastle disease

This disease is produced by an RNA paramyxovirus. It affects poultry and other birds and can cause severe damage because of its relatively rapid onset and high mortality of certain strains (Miller, 2018). Currently, Newcastle Disease Viruses (NDV) are classified into two categories depending on their virulence: virulent NDV and low virulence NDV. The second (lentogenic) only causes mild symptoms and is not a reportable disease, whilst the virulent NDV have a variable mortality depending on the type of strain (mesogenic or velogenic). Wild animals can be a reservoir of the low virulent NDV, which may mutate and become more virulent when transmitted to domestic birds. The animals can develop digestive, neural or respiratory symptoms along with other clinical signs such as lethargy. This disease may also be transmitted to humans on rare occasions (cases only reported for a few serotypes), especially after having a close contact with infected animals, but it is generally mild and self-limiting. However, the virus is not considered a food safety concern as there are no reported cases of infections after consuming poultry products. Currently, low virulent NDV are used in live vaccines to protect flocks. Virulent NDV is widespread in Asia, Middle East and Africa and endemic in certain areas of Latin America. The vaccination against Newcastle disease is mandatory in the EU, hence significantly lowering the risk of introducing this virus in the process via the sourced eggs. In fact, there is low risk that the eggs will be contaminated by this virus when laid by infected birds (Guittet et al., 1997). Consequently, the cell line was not tested for the presence of this virus but the results from the NGS transcriptomic analysis showed that there were no replicative viruses present in the cells (Confidential Appendix 6a).

Viruses from bovine species

The risk of introducing bovine viruses is considered to be low compared to avian viruses as the use of bovine-derived ingredients was minimal. Only FBS was used during isolation and adaptation of the cell line. The FBS was sourced from either Australia/New Zealand or the US and was tested in accordance with 9 CFR § 113.53(c) and the EMEA guidance (EMEA, 2003).

No viruses that could pose a risk to food safety have been identified. Among the most important viruses in the bovine species, the foot-and-mouth disease (FMD) virus can infect humans on rare occasions. FMD is listed as a notifiable disease of by the Australian Government Department of Agriculture, Fisheries and Forestry (GAFF). However, the GAFF notes that FMD is not transmitted to humans by eating affected meat. Nonetheless, the MCB was tested for the presence of bovine viruses (Confidential Appendix 6a). PCR and NGS results of the MCB show no replicative viruses are present in the cell line.

Viruses from porcine species

The risk of introducing porcine viruses is low compared to avian viruses as the use of porcine-derived ingredients was minimal. African Swine Fever (ASF), Classical Swine Fever (CSF) and swine Influenza are the main diseases, but reported as not being zoonotic (AHA, 2015, AHA, 2022) and hence, neither are of food safety concern. Nonetheless, for reassurance, the MCB has been tested for the presence of porcine viruses according to 9 CFR (US legislation). Porcine ingredients need to be either heat treated or undergo a pH treatment (<5 pH for minimum 2 hours) as per the pertinent requirements in the EU.

Viruses from Murine Species

Mouse embryonic fibroblast feeder cells were used in the early stages of cell isolation (Confidential Appendix 4) The feeder cells were gamma irradiated to render them inactive and incapable of replication. The efficiency of inactivation was confirmed by Giemsa staining. The gamma irradiation was also used to inactivate any potential viruses or pathogens.

Murine Leukemia Viruses (MLVs) are prototypical gammaretroviruses that can cause tumours and are classified into subgroups based on the species that they can infect (Arias and Fan, 2014). Xenotropic MLVs can infect human cells. MLVs are routinely monitored in laboratories working with murine cells as it is a widespread adventitious agent.

Even though the likelihood of MLVs being present in the duck cell line was low, and MLVs are not a food safety risk, the MCB was tested to confirm absence by PCR and NGS (Confidential Appendix 6a).

C.2.1.4. Recombinant Growth Factors

Recombinant proteins have been used at different stages of the production process. In the early stages of isolation, six recombinant growth factors were used from passage 0 to passage 16. Five of the growth factors were expressed in *E. coli*, which has a safe history of use in food production and does not raise and food safety concerns. One growth factor was expressed in human embryonic kidney cells (HEK293) cells. The HEK293 cells are not part of Gourmey's production process and were used a production platform to produce a purified human recombinant growth factor used as a raw material/reagent in the early stages of isolation of the cell line. The HEK293 cells are not present in the recombinant protein growth factor and therefore have never been in contact with Gourmey´s cell line. The growth factor expressed in HEK293 cells used in the early stages of isolation was a soluble interleukin-6 alpha (37.6 kDa) which was >98% purity confirmed by SDS-PAGE and HPLC. The protein was sterile filtered (0.2 micron filter) and presented in a lyophilised form. HEK293 cells in suspension typically have a mean cell diameter ranging from 14 µm to 16 µm. Therefore, HEK293 cells would not be present due to physical separation and the use of the 0.2 micron filter. Out of an abundance of caution and in the interest of transparency, Gourmey tested its duck cells for cross-contamination with other species including *Homo sapiens* at different time points (e.g. cells from the MCB and WCB), and the cells were found to be free of DNA contamination from other species further confirming the absence of contact and contamination of Gourmey´s cell line with HEK293 cells.

Further information is provided in Confidential Appendix 5a and Confidential Appendix 5b. The growth factors used in early isolation will not be present in the final cell biomass as each growth factor was used at a very low concentration and each has a specific half-life which ranges from 2 minutes to 48 hours. Moreover, the dilution factor by cell division from the passage number that the growth factors were removed (p16) to the passage number when the MCB was created (p162) would be 1.76E+77, and at the time of harvesting the cell biomass would be 1.26E+94 (calculations are presented in Confidential Appendix 5b hence, following

this logic, the likelihood of the growth factors being present in the final cell biomass is highly unlikely. As such, the use of these growth factors at the early stage of isolation is not considered a food safety concern.

Recombinant human insulin was used [redacted] but is not used in the culture media used to produce the cell cultured duck cells. The recombinant human insulin was produced in *Saccharomyces cerevisiae*, which has a safe history of use in food and food production. The half-life of insulin is approximately 4 to 6 min (Arbit and Kidron, 2017) and the dilution factor by cell division after switching to bovine insulin at time of MCB (p162) would be 6.32E+11 and at time of harvest would be 4.55E+28. Dilution factor by food-safe media with bovine insulin at the time of MCB production [redacted] would be 1E+09 and at time of harvest would be 1E+29. Hence, the likelihood of human recombinant insulin being present in the harvested cell biomass in negligible and as such, does not pose a food safety risk. Furthermore, bovine recombinant insulin that was used in the food safe culture medium used for the cell biomass production was not detected in three batches of harvested biomass.

Recombinant bovine insulin was used to create the MCB and WCB. It is also added to the food-safe culture medium during the cell expansion and proliferation stage of the biomass production process as it is required for cellular function and growth. Recombinant duck insulin is not currently available on the market and recombinant chicken insulin is produced at a much lesser scale compared to bovine insulin. Despite the bovine sequence used, receptors in duck cells recognize the bovine insulin due to its homology to the avian counterpart. The amino acid sequence of the recombinant bovine insulin has not been modified and is homologous to the native protein. The amino acid sequence for the recombinant bovine insulin is provided in Confidential Appendix 16b.

The recombinant bovine insulin used is produced in a genetically modified strain of *Pichia pastoris* (*Komagataella pastoris*). This yeast has a long history of safe use as a production strain in food and is not known to produce any secondary or toxic metabolites. In the EU, *Pichia pastoris* (*Komagataella pastoris*) is a QPS (Qualified Presumption of Safety) microorganism given that this species is not pathogenic or able to produce toxins (EFSA, 2020) with several strains used in the production of permitted processing. The genome of the production strain for the bovine insulin has been sequenced according to EFSA guidelines (EFSA, 2024). For the bovine insulin, data to support the absence of viable cells and host strain DNA was conducted in accordance with EFSA guidelines (2018). No viable cells were detected, and host strain DNA was below a 10 ng/g limit of detection (LOD) (Confidential Appendices 17 and 18).

As mentioned, residual insulin will be degraded by further processing and digestion. Based on the low levels of insulin, no concerns have been identified for the use of recombinant bovine insulin. The exposure to bovine insulin derived from cell cultured duck is further discussed in Section E.1.3. However, no safety limits have been established or identified for bovine insulin. In the unlikely event of bovine insulin being present in the cell cultured duck, it would be digested and absorbed as peptides and amino acids. Consequently, the use of recombinant bovine insulin in the production process does not introduce any food safety risks.

C.2.2. Chemical risks

Common chemical risks in the food industry (e.g., heavy metals, mycotoxins, dioxins and PCBs, etc.) are directly covered in the Food Safety Plan (please refer to Confidential Appendix 3). The Supplier Management Program and other prerequisites play an important role in the prevention of these contaminants and those that may be introduced by bad practices. This section focuses on the toxicological safety of certain compounds used in the media. Gourmey has developed a classification of the media ingredients depending on various factors:

To aid the risk assessment, the following classification criteria for the media ingredients has been applied:

- **Category A** - ingredients that have traditionally been used in food, are authorised food substances, nutrients or have been used in food with no limitations (limited by GMP).
- **Category B** - ingredients that have traditionally been used in food, are authorised food substances or commonly used substances but for which a toxicological or safety threshold has been established.
- **Category C** - ingredients with no history of safe use in food.

For ingredients belonging to Category A, no risk assessment is needed as these are considered safe. For Category B, a simplified risk assessment will be carried out to determine whether their presence in the product does not result in inadequate exposure above safe limits. The majority of media components used during each stage of production belong to Category A (e.g. amino acids, vitamins, etc.).

Finally, any ingredients under Category C will need to undergo a full safety assessment which will consist of the following:

- Characterization of substances: defining the identity of the substance (chemically defined, complex mixture), origin (mineral, animal, plant, fermentation), whether it is a normal constituent of the body and/or if it can be found in food in significant amounts in comparison with the intended use.
- Identification of public literature to retrieve information on its toxicological profile, distribution in food/body, toxicokinetics, adverse event reports or other topics related to safety.
- Assessment of its potential presence in the final product which may include mathematical models and/or experimental data. When no toxicological data can be retrieved, sufficient certainty should be provided that the ingredient is not present in the final product.

Below, a more extensive narrative is presented for ingredients belonging to Category B and Category C.

C.2.2.1. Chemically defined media used in early stages of isolation

During the early stages of isolation and adaptation, proprietary serum-free/animal-free media formulations were used. As such, it has not been possible to fully determine the exact composition of the medium as culture media suppliers will not disclose their formulations. However, certain patents are available that provide specific details on the formulation of chemically defined media (CDM) that are typically used in cell culture, and these serve as a good basis for determining the standard components that are used in CDM. For this exercise, patent 10/067382 (Lee et al., 2005) on chemically defined medium for culture mammalian cells was used as a reference on which to base the risk assessment.

The risk assessment for each component is presented in Confidential Appendix 5b.

Cells in culture are highly sensitive to their environment and are dependent on the culture medium to provide them with the nutrients required for normal cellular function. Trace metals are required to produce cofactors of enzymes that mediate metabolic pathways and regulate cellular metabolism and growth (Prabhu and Gadgil, 2021). Both deficiency and excess of trace metals can cause cellular function dysregulation, resulting an inhibition of growth and possible cytotoxicity (Deng and Wang, 2023). Homeostasis of trace metals in cells is maintained by metalloregulatory systems that control the labile pool of metal ions to favour binding of a preferred metal cofactor (Barwinska-Sendra et al., 2017, Foster et al., 2014, Prabhu and Gadgil, 2021). These systems import and mobilize metal ions under metal limiting conditions and uptake and export systems are achieved by transmembrane transport with the aid of different transporter families, to ensure relatively stable intracellular quota while they efflux and sequester metal ions by binding or storing under excess metal conditions. Stability of metal and ligand binding in biological systems follow the Irving–Williams series in the order $Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ with maximum stability with Cu^{2+} (Foster et al., 2014, (Williams and Fraústo da Silva, 2000). Consistent with this, the total amount of available metal ions like $Cu^{2+}$and Zn^{2+} in the cytosol are tightly regulated in cells compared to Mg^{2+} or iron (Fe^{2+}) (Williams and Frausto da Silva, 2000). This contributes to the binding of the correct cofactor and ensuing biological function. Changes in extracellular concentrations of trace metals can affect their own and possibly other trace metals' availability and homeostasis, and impact cell physiology (Prabhu and Gadgil, 2021). This means that the cells will only take up the nutrients and trace metals required to maintain cellular function, limiting the potential for bioaccumulation.

The trace metals and compounds without a history of safe use are used in very small amounts to minimize the risk of cytotoxicity and dysregulation of cell metabolism. Moreover, if these compounds were used in the culture medium during the early stages of isolation and adaptation, it can be reasonably assumed that they will have been washed out due to the exponential dilution effect and expansion of the cells over a significant period of time.

ESCs exhibit many intrinsic properties that enable them to proliferate extensively in culture compared to somatic cells such as fibroblasts. These properties include their pluripotency, high telomerase activity, and accelerated cell cycle that enables rapid proliferation.

The accelerated cell cycle of ESCs facilitates rapid proliferation, maintaining the cells' undifferentiated state across numerous passages. The inherent pluripotency of ESCs, coupled with their high telomerase activity, ensures that they can replicate without succumbing to the telomere shortening that typically limits cellular lifespan (Hayflick limit). This characteristic, combined with their genetic stability, contributes to the longevity of ESC cultures, enabling them to undergo many more passages than cells such as fibroblasts.

Because ESCs can undergo more passages than primary cell lines, it translates into a higher dilution factor for any accumulated substances within the cell culture over the production cycle. Each passage involves splitting the cells and introducing them to fresh media, effectively diluting metabolic byproducts, extraneous compounds from media, and cellular waste. This process repeated more frequently due to the number of passages ESCs are subjected to.

The calculations for the dilution factors are presented in Confidential Appendix 5b.

Further, certain trace metals were measured in the harvested cell biomass (aluminium, barium, cobalt, tin) that are typically used in CDM and were found to be below the method LOQ (Table 8, Confidential Appendix 8) supporting the hypothesis that trace metals that may have been used in the proprietary medium used in the early stages of isolation would be diluted out due to the exponential dilution effect and expansion of the cells over a significant period of time.

Based on the trace amounts of non-food grade components used, cellular function and metabolism, and the dilution factor, the use of proprietary medium in the early stages of isolation are not considered a food safety concern.

C.2.2.2. Category B Media Components

The media inputs used in the early stages of isolation until creation of the cell banks are heavily diluted in the later process of biomass production and are expected to be present in residual amounts. Therefore, only the category B media components which are used in the process from Master cell bank creation until Biomass harvest are further discussed.

.

C.2.2.2.3. 4-Aminobenzoic Acid or P-aminobenzoic acid (PABA)

4-aminobenzoic acid or para-aminobenzoic acid (PABA) is an organic compound essential for the growth of various yeasts, bacteria and plants but it is a non-essential nutrient for humans. PABA is an intermediate involved in the synthesis of folic acid in these organisms and has also been identified as a coenzyme Q precursor. Due to its crucial role in microorganisms and plants, PABA is naturally present in various foodstuffs such as brewer's yeast, mushrooms and whole grains, and is synthesized by microbiota in the human intestinal tract. PABA is currently being marketed as a dietary supplement (FDA, 2023) at concentrations up to 500 mg per daily dose and above.

PABA is rapidly absorbed in the intestine and excreted via urine mainly as 4-aminohippuric acid by glycine conjugation, N-acetylated products or glucuronide conjugates. A very small amount is excreted as free PABA (Tabor et al., 1951, Krátký et al., 2019).

Based on Lazar's predictions (Maunz et al., 2013), the substance is not carcinogenic or mutagenic and the Lowest Observed Adverse Effect Level (LOAEL) was reported to be 222 mg/kg BW/day in rats and 12.1 mg/kg BW/day in humans (Figure 2). An unpublished study in rats given 1,200 mg/kg BW/day of PABA for 108 days reported no adverse effects at this dose. Hence, NOAEL was considered to be greater than 1,200 mg/kg BW/day (EC, 2006).

Figure 2. Lazar's prediction of p-aminobenzoic acid's toxicological profile (August 2023)

A study performed by (Chang and Hu, 1996) reported the oral toxicity of PABA when administered to rats through drinking water. Over a period of four weeks, no significant effects on body weight gain or the weight of vital organs like the liver, kidney, and spleen were observed in rats given PABA. Rats consuming 0.5% and 1% PABA in their water exhibited increased PABA levels in the liver, kidney, and blood, while a lower concentration of 0.1% PABA did not have a noticeable impact. The results of this study suggest that PABA can accumulate in the body to a certain degree without causing apparent toxicity, even at relatively high concentrations in drinking water. Additionally, PABA demonstrated some antioxidant properties that could potentially protect against membrane damage in the liver.

In a report from the European Commission Health & Consumer Protection Directorate-General, a series of toxicological data on PABA are reported. Studies on the acute toxicity of PABA in various animal species indicated that high doses could lead to toxic effects such as gastro-enteritis with haemorrhages and acute necrosis of the liver in mice, rats, and dogs. In humans, cases have been reported where high-dose PABA administration (3 g four times daily) for a period of four weeks resulted in symptoms such as myalgias,

generalized weakness, fever, and hepatic injury. These symptoms and hepatic dysfunction reversed upon discontinuation of PABA. Another case involved hepatotoxicity after oral intake of PABA at a dose of 12 g/day for approximately two months. Although PABA did not show adverse effects after repeated oral administration in some studies, these case reports highlight the potential for hepatic injury with prolonged high-dose usage in humans. Furthermore, a case of chronic tubulointerstitial nephritis in a 19-year-old woman was potentially linked to long-term oral PABA use for vitiligo treatment, suggesting a need for caution with long-term oral consumption (EC, 2006).

PABA is added as a nutrient to the culture media used during the proliferation and expansion phase of production. PABA will consequently be consumed by the cells hence, this compound is not expected to be present in the cultured duck ingredient. Considering that PABA is consumed in dietary supplements, the concentration in the media is low and it is utilized by the cells, PABA is not considered to be a food safety risk.

C.2.2.3. Category C Media Components

The media inputs used in the early stages of isolation until creation of the cell banks are heavily diluted in the later process of biomass production and are expected to be present in residual amounts. Therefore, only the Category C media components which are used in the process from MCB creation until biomass harvest are further discussed.

C.2.2.3.1. Recombinant Bovine Insulin

Recombinant bovine insulin was used to create the MCB and WCB. It is also added to the food-safe culture medium during the cell expansion and proliferation stage of the biomass production process as it is required for cellular function and growth. Recombinant duck insulin is not currently available on the market and recombinant chicken insulin is produced at a much lesser scale compared to bovine insulin. Despite the bovine sequence used, receptors in duck cells recognize the bovine insulin due to its homology to the avian counterpart.

The recombinant bovine insulin used is produced in a genetically modified strain of *Komagataella pastoris*. This yeast has a long history of safe use as a production strain in food and is not known to produce any secondary or toxic metabolites. Several GRAS notices of food ingredients produced by this microorganism can be found in the GRAS notice inventory (GRN 1025, 1001, 938, 737, 204, etc.). Moreover, in the EU, *K. pastoris* is a QPS (Qualified Presumption of Safety) microorganism given that this species is not pathogenic or able to produce toxins (EFSA, 2020). Further information on the bovine insulin and an example of CoA is presented in Confidential Appendix 16.

Hormones are produced by the cells as part of their normal cellular activity. These compounds play an essential role in cell metabolism and are needed to promote and maintain healthy and stable growth. As part of their metabolism, cells will synthesize hormones and other large proteins within a normal activity range. These proteins are expected to be present in the cultured duck in the same way that they are present in conventional meat and other animal-derived products. Bovine recombinant insulin is the only hormone added to the food-safe culture medium used for cell proliferation and expansion in the bioreactor. Insulin has a short half-life of approximately 4 to 6 min (Arbit and Kidron, 2017), so it is unlikely to be present in cell cultured duck. Nevertheless, the presence of bovine insulin was tested in five batches of which was found to be below the limit of quantification (LOQ) in three batches (LOQ) (2 µg/g) and below the limit of detection (LOD) (0.5 µg/g) in two. Ingested insulin will face various barriers limiting its absorption as dietary proteins do not normally cross the intestinal epithelium intact and must first be broken down into small peptides and amino acids. This route of protein absorption destroys all physiological activity of protein and explains why typical oral bioavailability of proteins is usually less than 1–2%. Protein digestion by proteases (e.g., pepsin) begins in the stomach and is complemented by many different enzymes located throughout the gastrointestinal (GI) tract. Trypsin, chymotrypsin and carboxypeptidases from the pancreas are located in the small intestinal lumen. The remainder of the degradation occurs at the brush-border membrane by various peptidases, or within the enterocytes of the intestinal tract. There also exists an insulin specific cytosolic enzyme (insulin-degrading enzyme) ubiquitously expressed with multiple functions (Pivovarova et al., 2016, Carino and Mathiowitz, 1999).

In the GI tract, exogenous insulin enzymatic digestion occurs mainly by insulysin (previously called insulinase) and pancreatic enzymes. The first is present in cytosolic fraction of human ileal and colon tissues and on the brush border membrane of enterocytes and present an insulin degrading activity. It cleaves insulin at a limited number of bonds (two bonds in the A-chain and seven bonds in the B-chain), although highly specific to insulin, insulin-degrading enzyme (IDE) also degrades insulin-like growth factors. The main hypothesis on the cellular insulin degradation process in GI tract is that after binding to its receptor (receptor-mediated endocytosis), insulin is internalized by endocytosis in cells wherein it can be degraded (Yamamoto et al., 1990, Bai et al., 1996, Duckworth et al., 1998, Chang and Bai, 1996). Pancreatic enzymes like trypsin, chymotrypsin and carboxypeptidases also break down insulin as it passes through the GI tract. Trypsin and α-chymotrypsin, the main proteolytic enzymes secreted by the pancreas into the intestinal lumen, cleave the insulin molecule at 2 and 7 sites, respectively, while carboxypeptidases degrade the insulin by cleaving the carboxy-terminal residue of the B chain (Duckworth et al., 1998, Marschütz et al., 2000).

In addition to the above, insulin is poorly absorbed in the GI tract. In fact, various studies have focused on the improvement of oral absorption in diabetes treatment. Until now, no efficient oral medication is on the market, and several trials are still investigating how to increase the insulin uptake when administered orally. Studies show that insulin's greatest permeability is in the distal jejunum and the lowest in the duodenum (Bendayan et al., 1990, Schilling and Mitra, 1992). Nevertheless, insulin is poorly absorbed by the intestine epithelium due to insulin’s hydrophilicity and large dimensions, as well as strong insulin degradation due to the pH and enzymes in the stomach and small intestine. This results in no observable changes in the insulin blood level. This has been further confirmed in many *in vivo* studies on rats as recently reviewed by Iyer et al. Iyer et al. (2022) and Fonte et al. (2013). In fact, oral doses of up to 50 UI/ kg BW have shown no changes in the blood glucose levels. Only in the case of newborns, the absorption of molecules such as insulin is significant, as the intestinal permeability is greater than in adults and the proteolytic activity is lower. Milk (whether human or bovine) naturally contains insulin, transferrin and other large proteins with physiological activity. However, the presence of these molecules has not been linked to any physiological effect in the literature, indicating that these molecules are not entering into the bloodstream.

Insulin concentration in bovine milk during the first period of lactation goes from 327 ng/mL in the first milking to a stable value of 46 ng/mL at day 7. In human milk, insulin concentration is approximately 4 nM in the colostrum and drops to 0.46 nM in mature milk (Jouan et al., 2006, Koldovský, 1994, Aranda et al., 1991, Hazum, 1991, Read et al., 1984). No detailed data on insulin content in meat were found. As insulin is not stored within the skeletal muscle cell, the insulin in meat (mainly present in the residual blood and interstitial volume in meat) might represent a very low - or even negligible - fraction. In pig, sheep and cattle, the residual blood content of lean meat is 2 to 9 ml/kg muscle (Warris, 2009). For a plasma level of 10µU/mL, considering a maximum residual blood content of 9 ml/kg, it means that 1kg of meat contains approximately 90 µU of insulin. Considering that one unit of bovine insulin is equivalent to approximately 0.03891 mg, the estimated content of insulin in red meat is 3.12 mg/kg. Although no information on the residual blood present in poultry or duck meat was found, it can be assumed to be less. Consequently, the levels of insulin may also be lower than in red meat. However, it should be noted that the concentration in the muscle is negligible as insulin is rapidly metabolized by the cells.

It should be noted that growth factors are naturally present in milk, meat and other food products (including vegetables). These food products have a long history of safe consumption and are frequently consumed as part of a normal diet. With the exception of thyroid hormones, hormones are denatured and hydrolysed during digestion, hence not having a direct effect on the blood levels of their human counterparts (Palacios et al., 2020).

C.2.2.3.2. Pluronic Acid

Pluronic® F-68 (also known as poloxamer 188) is used in the culture medium to protect the cells from shear stress and cell adhesion caused by agitation in the bioreactor during the growth and amplification phase of production.

Pluronic® F-68, poloxamer 188 and pluronic acid are used interchangeably throughout this dossier.

Pluronic® F-68 is a non-ionic, polyoxyethlyene-polyoxypropylene block polymer (average molecular mass of 8,400 g/mol). Pluronic® is one of the trademarks under which poloxamers are commercialized. Poloxamers are composed of polypropylene oxide (or polypropylene glycol or PGG) and polyethylene oxide (or polyethylene glycol or PEG) block polymers and are synthesized at high temperature and pressure from propylene glycol to which propylene oxide is added followed by ethylene oxide in the presence of an alkaline catalyst (CIR, 2008). The digits in Pluronic® and poloxamer indicate the molecular mass (first digit or first two digits) and the percentage of polyethylene glycol (PEG) (or ethylene oxide) (second or third digit, multiplied by ten). Although other Pluronic® and poloxamers with different codes are included as synonyms to Pluronic® F-68 or poloxamer 188 (molecular mass ca. 8,400 g/mol) on various websites, they have different molecular mass and proportion of polyethylene glycol.

The characteristics of poloxamers are determined by the length of the polymer blocks. These non-ionic surfactants have multiple applications in cell culture, cosmetics, pharmaceuticals, foods and chemicals. Poloxamers are amphiphilic compounds, meaning they have both hydrophilic and hydrophobic segments. This dual nature enables versatile interactions with cell membranes and a range of biological and chemical substances. These interactions stabilize interfaces and reduce surface tension which can enhance the solubility and stability of various compounds. In cell culture, poloxamers like Pluronic® F-68 align at liquid interfaces to form protective barriers around cells, significantly mitigating mechanical stress, shear forces, and reducing cell adhesion. The mechanical properties exhibited by these polymers, therefore have proved beneficial in mammalian and insect cell cultures.

History of use of poloxamers

Several poloxamers (poloxamer 105 to 407) are listed as permitted direct and indirect food additives in the FDA Substances Added to Food Database for use as processing aids in flavourings, wetting agents, defoamers, defeathering agents and dough conditioners at concentrations between 0.05-0.5% (w/w). Poloxamer 188 is not listed specifically but falls in range of the size of polymers that are permitted for use.

Table 16. Summary of poloxamers approved for use in food

Poloxamer	CAS No (molecular weight)	Uses	Regulated limit	Reference
Poloxamer 105	977057-63-8 (1,900)	Indirect food additive. Sanitizing solutions.	Should not exceed 0.5% by weight of the flour used. CEDI: 27.4 µg/kg bw/d CDC: 548 ppb	21CFR178.1010 21CFR172.808
Poloxamer 181	977057-68-3 (2,000)	Indirect food additive. Sanitizing solutions.	CEDI: 8µg/kg bw/d CDC: 160 ppb	21CFR178.1010
Poloxamer 331	977057-83-2 (3,500-4,125)	Food additive permitted for direct addition to food. Multipurpose additive	The finished beverage or fruit juice drink will contain not in excess of a total of 10 ppm of the dioctyl sodium sulfosuccinate-block copolymer combination.	21CFR172.810
Poloxamer 338	977057-87-6 (14,000)	Dough strengthener, leavening agent, processing aid, stabilizer or thickener, surface-active agent	No regulatory limit established	Substances added to food
Poloxamer 407	77057-91-2 (9,760-13,200)	Dough strengthener, leavening agent, processing aid, stabilizer or thickener, surface-active agent.	The finished beverage or fruit juice drink will contain not in excess of a total of 10 ppm of the dioctyl sodium sulfosuccinate-block copolymer combination.	Substances added to food 21CFR172.810

Bw/d = body weight per day
CEDI = Cumulative estimated daily intake
CFR = Code of Federal Regulations
CDC = Cumulative dietary concentration
PPB =Parts per billion
PPM = Parts per million

Poloxamer 188 is listed under 21 CFR § 310.545(a)(12)(iii) as a stool softener. Poloxamer 188 is also listed as an inactive ingredient (excipient) in 15 approved drug products administered via the oral route with the maximum daily exposure values ranging from 6 – 200 mg. The use of poloxamer 188 as an inactive ingredient is further discussed below.

Oral toxicity

In a study reported by (Comai and Sullivan, 1980), as cited by Cosmetic Ingredient Review (CIR) Expert Panel (2008), female Charles River CD strain rats fed a high-fat diet with added Pluronic F-68 showed no significant impact on feed intake, faecal-fat elimination, or dietary fat absorption compared to controls.

Leaf (1967) performed oral sub-chronic toxicity studies in rats. Animals were given a diet with 0%, 3% or 5% (by weight) for a period of 90 days. At the lowest doses, animals were asymptomatic, but as the dose increased, the rats showed signs of mild sedation, which increased in severity over time. Necropsy was performed on the animals that died and marked engorgement of the lungs, stomach distention and massive vascular dilation of the intestines was noted. In acute toxicity testing, the oral LD50 was determined to be >15 g/kg. In the 90-day sub-chronic portion, a 3% inclusion level, equivalent to 1,500 mg/kg BW/day, did not produce systemic toxicity and was identified as the NOAEL. In another study, a small group of dogs (12) received a capsule with 0, 0.05 or 0.1 g/kg poloxamer 188. No adverse events were reported in this study (Leaf, 1967). The same author also conducted a 6-month sub chronic toxicity study in 45 rats fed and poloxamer 188 at 0%, 3% and 5%. During the study period two animals died from the 3% group and 14 animals from the 5% group. The deaths were attributed to infection and inanition. Necropsy performed on the dead animals did not show signs of adverse effects. In a further two-year chronic oral toxicity study with rats (number of animals not reported) the groups were administered a diet with 0%, 3%, 5%, 7.5% poloxamer 188 for a period of 24 months. The control animals showed a higher mortality rate compared to the treatment groups. A reduction in growth was also observed in the 7.5% group. However, no other adverse events were reported during the trial or after the post-mortem examination (Leaf, 1967).

Additionally, purified poloxamer 188 has been shown to be safe and well tolerated at doses as high as 200 mg/kg as a 1-hour bolus infusion (Luchtman-Jones et al., 2000). Moreover, the Acceptable Daily Intake (ADI) of Kolliphor® P188 (another tradename used for poloxamer 188) has been reported to be 200 mg/day in adults (Halder et al., 2021, Singh-Joy and McLain, 2008).

The safety of poloxamer 188 for use as an oral excipient in pharmaceuticals has been reviewed by the FDA. An “oral excipient” is an inactive substance formulated with an active pharmaceutical ingredient (API) to facilitate the manufacturing process, enhance stability, or improve solubility. In this context, poloxamers do not provide any therapeutic effect and are not classified as APIs themselves; instead, their function is strictly technical. The FDA routinely reviews the safety of both active and inactive components in new or supplemental drug applications, which includes analysing potential toxicity and exposure profiles for oral excipients. Consequently, because these excipients—such as poloxamer 188—are reviewed for long-term safety alongside the API for prolonged oral administration at specified levels for repeated oral intake, this regulatory acceptance strongly indicates that lower-level exposures in a food-based application are unlikely to carry an additional risk.

In a pharmacological review for fenofibrate containing Poloxamer 188 in the tablet formulations, an FDA approved treatment that is used to treat hypertriglyceridemia, primary hypocholesterolemia and mixed dyslipidaemia administered to patients long-term (FDA, 2007), the FDA reported oral NOAELs of 2,500, 100 and 1.4 mg/kg BW/day for rats, dogs and humans, respectively. The NOAEL reported in humans corresponds to the human dose. Poloxamer 188 concentration was reported to be above the established limit of 18 mg/day for oral tablet formulations as listed in the FDA Inactive Ingredient Guide. The concentrations of poloxamer in the oral tables were, however, not disclosed. In a posterior publication reviewing the safety of an oral pharmaceutical (FDA, 2017), the FDA reported the same NOAELs and derived the human equivalent dose from the NOAEL reported in dogs (the most sensitive species). Although older publications such as Leaf (1967) originally offered foundational toxicological insights for poloxamers, more recent reviews and regulatory documents collectively confirm the low toxicity profile of pluronic acid when administered orally. Among these sources are FDA-approved oral drug products in which poloxamers (including poloxamer 188) serve as inactive excipients, with daily exposures often reaching 1,800 mg or more. The maximum permitted

concentrations of poloxamer 188 in oral formulations (capsules, granules, powder for suspension, tables, etc.) are illustrated in Table 17 below. These uses are documented in the publicly available FDA Inactive Ingredient Database (IID) and arise from the FDA's broader pharmacological reviews of poloxamer-based products.

Table 17. Maximum Daily Exposures reported in the FDA's Inactive Ingredient Guide database

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Daily Exposure (MDE) (mg)
POLOXAMER 188	ORAL	CAPSULE	691397134	LQA7B6G8JG	151
	ORAL	CAPSULE, DELAYED RELEASE	691397134	LQA7B6G8JG	18
	ORAL	CONCENTRATE	691397134	LQA7B6G8JG	3
	ORAL	GRANULE	691397134	LQA7B6G8JG	81
	ORAL	GRANULE, FOR SOLUTION	691397134	LQA7B6G8JG	624
	ORAL	POWDER, FOR SOLUTION	691397134	LQA7B6G8JG	500
	ORAL	POWDER, FOR SUSPENSION	691397134	LQA7B6G8JG	30
	ORAL	SOLUTION	691397134	LQA7B6G8JG	1,800
	ORAL	SUSPENSION	691397134	LQA7B6G8JG	1,600
	ORAL	SUSPENSION, EXTENDED RELEASE	691397134	LQA7B6G8JG	6
	ORAL	TABLET	691397134	LQA7B6G8JG	100

Absorption Metabolism Distribution and Excretion (ADME)

Data derived from intravenous applications across various animal species and humans consistently show that the compound is rapidly eliminated through the urine with minimal metabolic alteration (CIR Expert Panel, 2008).

In human studies where Pluronic F-68 was administered intravenously at doses ranging from 10 to 90 mg/kg body weight per hour for periods of 24 to 72 hours, between 72 to 94% of the dose was excreted via the urine. The clearance mechanism, while not definitively established, is thought to occur through glomerular filtration. This hypothesis is supported by the non-ionic nature and large macromolecular structure of Poloxamer 188, which lacks an electrical charge, facilitating its passage through the glomerular filter. However, the substantial molecular size of Pluronic F-68 may impede its filtration to a certain extent (Jewell et al., 1997). Additionally, the pharmacokinetic properties of Pluronic F-68, including its clearance, elimination, and volume of distribution, appear to be consistent across different infusion rates, indicating a lack of dependence on the rate of infusion.

In a further study, Grindel et al. (2002) conducted an investigation into the distribution, metabolism, and excretion of Pluronic F-68 in rats, dogs, and humans. Their findings indicated that 75 to 95% of the administered intravenous dose was excreted in the urine, with approximately 90% noted in humans, mostly as the unchanged polymer. Less than 5% of the dose was excreted via the faeces. A single metabolite characterized by a molecular weight of 16,000 Da and a block copolymer structure was identified at low concentrations in all species tested, supporting the finding that less than 5% of the administered dose underwent metabolic transformation. Tissue distribution was mostly confined to extracellular fluid and select organs (kidneys, lymph nodes, liver, spleen, urinary bladder, gastrointestinal tract) with minimal absorption by red blood cells. The metabolism of Pluronic F-68 was limited, accounting for less than 5% of metabolism, and elimination from the body was primarily through renal excretion, with the 48-hour infusion doses being cleared from all species roughly one week after the end of administration. The distribution, metabolism, and excretion patterns observed for Pluronic F-68 align with those expected for other non-ionic block copolymers that share similar physical and chemical characteristics with poloxamer 188.

When poloxamer 188 was given intravenously, studies revealed dose-dependent pharmacokinetic parameters in rats, achieving a steady state within 46 hours, whereas in dogs, a steady state was reached after 7 days with a mean plasma clearance between 49.4 and 87.9 mL/hour. In humans, the peak plasma concentration of Pluronic F-68 occurred within 1 hour of intravenous administration. Additional research by

Gibbs and Hagemann (2004) indicated that while Pluronic F-68 predominantly remained within the extracellular fluid, it was detectable at low concentrations mainly in the liver, lungs, and kidneys of dogs' post-administration. Approximately 26% of Pluronic F-68 in plasma was bound to the albumin fraction, with the remaining 74% circulating freely. Considering that poloxamer 188 is primarily eliminated via the urine with minimal metabolism when administered intravenously, oral ingestion would generally result in even lower systemic bioavailability due to partial or minimal absorption in the gastrointestinal tract. Therefore, systemic toxicity is expected to be further reduced relative to intravenous exposure. Considering that poloxamer 188 is primarily eliminated in urine with minimal metabolic alteration when administered intravenously, it is likely that oral exposure would result in equal or greater excretion, but with reduced systemic toxicity as it is not expected to be absorbed.

Safety of pluronic acid in cell cultured duck

In the production of cell cultured duck, pluronic acid (e.g. poloxamer 188) functions as a processing aid consistent with 21 CFR § 170. It is introduced during manufacturing to reduce shear stress and cell adhesion but has no nutritional or organoleptic role in the final product. A maximum of 500 ppm (0.05%) for poloxamer in Gourmey's cell cultured duck is proposed as the specification.

Even under a conservative scenario where an individual ingested 1 kg of cell cultured duck in a single day, total poloxamer intake would be 500 mg. If an individual were to consume 1–1.5 kg, the total would be 1,000–1,500 mg, which equals or falls below the 1,600–1,800 mg daily exposures found in certain FDA-approved oral poloxamer solutions. This comparison underscores that the residual poloxamer in our product is likely to be well within a safe margin of exposure. By analogy, if poloxamer 188 is safe for chronic, repeated ingestion as an inactive ingredient in oral drug products, its incidental presence at 500 ppm in cell cultured duck similarly should present no meaningful safety concern.
The publicly available toxicological and safety data from studies involving rats, dogs, and humans suggest that oral exposure to Pluronic F-68 at moderate to high levels is unlikely to cause adverse effects. In long-term feeding studies with rats and dogs, no specific toxic effects were observed, except for diarrhea at highest doses. Using the NOAEL of 1,500 mg/kg bw/day (CIR Expert Panel, 2008) an acceptable daily intake (ADI) was derived by applying a 100-x safety factor to account for inter-species and intra-species variation. Following this rationale, an ADI of 15 mg/kg bw/day has been determined. This ADI is roughly equivalent to 900 mg/day for a 60 kg adult (CCC001, 2022).

The exposure from current uses has been estimated to calculate the overall exposure from all dietary and other sources. Non-dietary sources include cosmetics and medicines. Cosmetics have not been considered as the compound is not absorbed through skin due to its high molecular mass. The intake derived from medicines containing pluronic acid could not be estimated based on the lack of available data. This limitation in the assessment is not expected to have a significant impact as the exposure derived from medicines containing pluronic acid is likely to be marginal.

A detailed exposure assessment to the substance derived from the consumption of cell cultured duck is presented in Section E.1.3.1. Pluronic acid Figure 3 shows the predictive toxicological profile obtained with Lazar Toxicology Prediction, an *in silico* open-access software endorsed by different entities. The range of the results obtained with this program support the NOAEL obtained in the reproductive and system toxicity assay.

It should be noted that poloxamer 188 is a large molecule with an approximate molecular weight of 8,400 Da. Overall, the safety of long polymers is mainly defined by the safety of low molecular weight fractions (LMWF) present in the product and/or formed after digestion or food processing. The literature does not provide much information on the presence of LMWF and the effects of digestion and processing on its structure and integrity are unclear. An article recently published (Bandyopadhyay et al., 2022) analysed a batch of poloxamer 188 with low performance and reported the presence of low molecular weight species in the batch. These were identified as polypropylene oxide (or PEG), but the molecular weight is not discussed in the abstract of the article. The homopolymers PEG and PPG (propylene glycol) have a solid safety profile. Only PEG and PPG species with a molecular weight greater than 400 Da are authorised in the EU for use in food (excluding FCM) based on the acute toxicity showed by species below 400 Da. Neither of these two monomers are

genotoxic or carcinogenic. Poloxamer 188 is not a reactive molecule based on its structure and consequently it is safe to establish a read-across approach from its monomers.

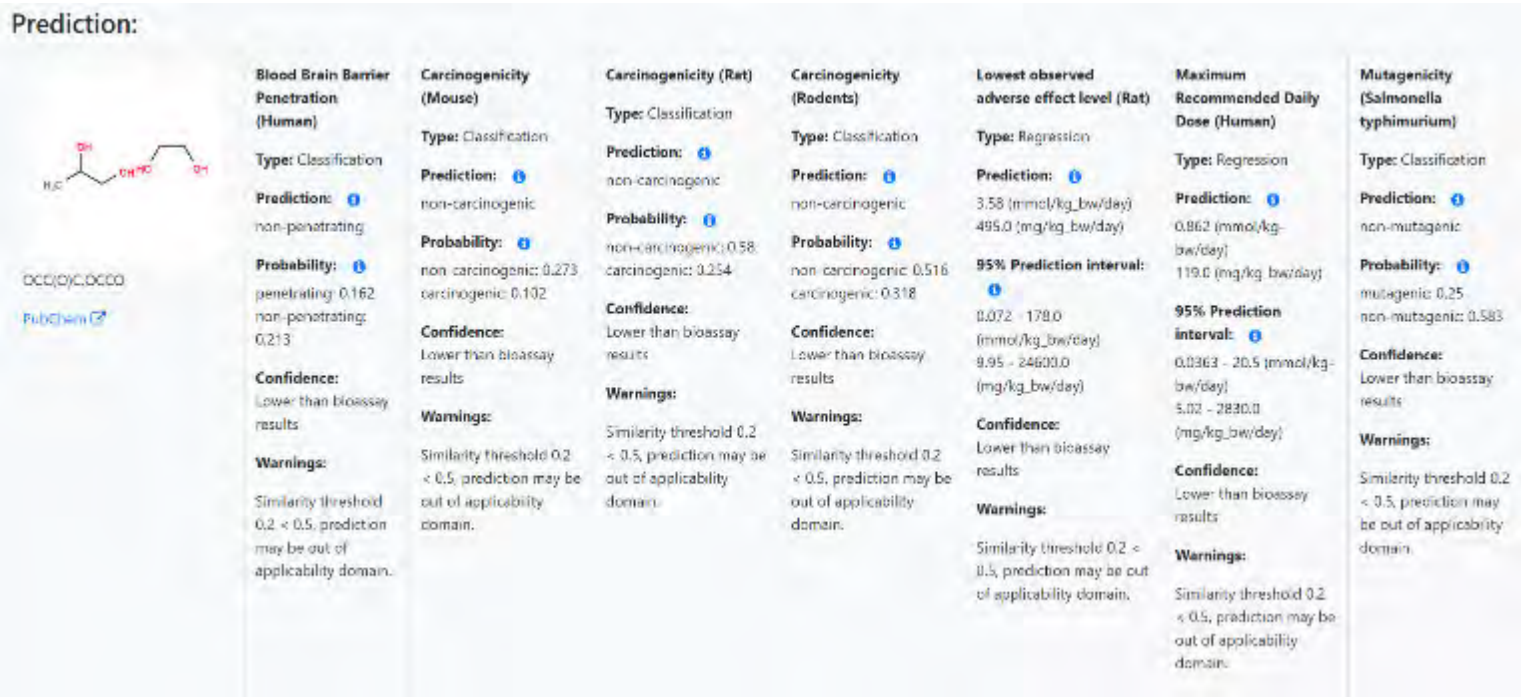

Figure 3. Lazar's prediction of Pluronic acid F-68's toxicological profile (February 2024)

In summary, FDA approvals for poloxamer use in oral pharmaceuticals as an inactive excipient, alongside publicly available toxicological data (including those outlined by Singh-Joy and McLain for various exposure scenarios) and the analytical data and exposure calculations indicate that its intended use in the production of cell cultured duck as a processing aid poses no meaningful safety concerns for the consuming public.

Additionally, ToxTree was used to determine Cramer class of Pluronic acid. The program could not adequately classify the compound based on its complex structure. Recreating the 3D structure of large, linear polymers is complex and consequently the TTC approach could not be applied to this substance.

C.2.2.3.2. Dimethyl sulfoxide (DMSO)

Dimethyl sulfoxide (DSMO) is commonly used as a cryopreservative in cell culture and has been extensively used as a solvent vehicle in *in vitro* genotoxicity assays.

The Australian Therapeutic Goods Administration (TGA, 2018) reports DMSO to be of low toxicity and not expected to be genotoxic, carcinogenic nor have reproductive or developmental toxicity although it may considered an eye and skin irritant at concentrated doses.

In mice, Caujolle et al. (1967) observed weight loss during the first days of the experiment in animals given 5 g/kg BW/day for 20 days. This effect was corrected shortly after, and the treatment group showed no significant difference to the control group. In rats given 0, 2 or 5 g/kg BW/day for 45 days, the researchers did not observe any adverse event or abnormality during histopathological examination in the 2 g/kg BW/day. However, the 5 g/kg BW/day showed slight weight loss and necrosis in the liver tissues with inflammation and irritation of the portal spaces. The authors defined DMSO as non-teratogenic when administered orally based on different studies in mice and rats.

Smith et al. (1967a) gave doses of 1, 3 and 10 g/kg BW/day by gavage to rats for 59 days and only observed a slight and reversible weight reduction similar to that observed by Caujolle et al. (1967) in their studies. No

other gross abnormalities were reported. However, the study could not be retrieved and the information available is limited. Their work also mentions a study conducted by Sommer and Tauberger in 1964 where the researchers gave 0.4, 7 and 14.1 g/kg BW/day to rats for 13 weeks. Animals in the 0.4 and 7 g/kg BW/day showed atrophy of the spleen, and no other adverse events were reported, whilst deaths were observed in 90% of the animals in the 14.1 g/kg BW/day group.

Oral toxicity studies were described by Noel et al. (1975) and were conducted on rats and dogs, while cutaneous toxicity testing was performed on rabbits and pigs using a 50% aqueous solution of DMSO administered by stomach intubation. Although there were some slight changes in body weight and blood composition, as well as increased diuresis, the main negative effects were detected in the cutaneous tests.

In a study in dogs carried out by Rubin and Barnett (1967), 12 beagle dogs (6 females and 6 males) were given 0, 2.5, 5, 10, 20 or 40 g/kg BW/day by gastric intubation for 23 weeks (5 days a week). Withdrawal from the administration happened at 23 weeks and the dogs were monitored for an additional period of 31 weeks. The two higher dose groups did not tolerate the doses. Ophthalmoscopic changes were seen in all doses, but the effect was very slight in the lowest dose group. The effects were only partially reversible after withdrawal. Histological examination was only performed on ocular tissues, hence other potential signs of toxicity were investigated. These results were similar to those reported by Smith et al (1967b) and Kleberger (1967), who concluded that a dose of 2.5 to 10 g/kg BW/day resulted in ocular damage, vomiting and discomfort when given for a period of 7 to 14 weeks.

In addition, the European Chemicals Agency and other agencies also found that repeated dose toxicity studies performed by different routes of administration and with several mammalian species have also shown that DMSO produced only slight systemic toxicity. More precisely, with the exception of a decrease of the body weight gain and some haematological effects, which could be secondary to an increased diuresis, at very high dose levels, the most common finding observed in these studies is changes of the refractive power of the lens. Species in which such lens alterations readily develop include the rat, rabbit, dog and pig, while primates are not sensitive. Clinical signs of systemic toxicity and the alterations of the lens have never been observed or reported in clinical and epidemiological studies performed in humans, even after exposure to high dose level (1,000 mg/kg BW/d for 3 months) or for a long period of time (up to 19 months). The NOAELs by oral and dermal routes in primates are 2970 and 8910 mg/kg BW/d, respectively (Usepa et al., 2006, Therapeutic Goods, 2021, ECHA, 2023).

Although DMSO has not been approved for oral administration, it is considered safe to consume orally within the limited concentration found in regulated pharmaceutical formulations. Moreover, animal studies have also shown that DMSO has a high acute toxicity threshold (LD50), indicating that the overall toxicity is low. As such, due the small quantities used during cryopreservation and the dilution effect from vial thaw to cell expansion, it is reasonable to conclude that the exposure to DMSO from cultivated duck is negligible. Consequently, a quantitative exposure assessment was deemed unnecessary.

C.2.2.2.3. Ethanolamine

Ethanolamine (EA) is a naturally occurring organic compound which is essential for the formation of cell membranes as a precursor of cephalins such as phosphatidylethanolamine, one of the most abundant phospholipids in cell membranes. Hence, it is naturally present in food. EA is also a degradation product of serine. FBS provides the cells a source of EA, hence a replacement is needed when FBS is removed from the media (Patel and Witt, 2017). Moreover, EA is present in lecithin, defined as “a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids” in 21 CFR § 184.1400. EA is also a permitted adjuvant substance for use in paper and paperboard in contact with food.

EA is not genotoxic or mutagenic based on different *in vitro* assays reported in the literature (CIR, 1983a, Council, 2007, Knaak et al., 1997).

A study by Knaak et al. (1997) examined the oral toxicity of EA and other alkanolamines in different animal species. The acute oral LD50 for EA was 2.74 g/kg, indicating a comparatively low level of toxicity. Rats that

were orally administered EA for a period of 30 days, with dosages ranging from 160 to 2,670 mg/kg/day, exhibited changes in the weights of their liver and kidneys at dosages of 640 mg/kg/day or above. Significantly, mortality was observed in rats when administered a dose of 1,280 mg/kg/day. Nevertheless, no discernible effects related to treatment were observed in dogs, even when administered doses of up to 22 mg/kg/day for a duration of 2 years. These findings suggest that there are differences between species in terms of how they respond to EA, with rats showing more noticeable effects, especially at higher doses.

In 1951, Smyth et al. conducted a 90-day study with rats fed a diet containing 0, 320, 640 or 1,280 mg/kg BW/day of EA. A total of 10 rats were allocated to each treatment group. The authors reported increased liver and kidney weights in the 640 mg/kg BW/day group and higher mortality in the 1,280 mg/kg BW/day group. The NOAEL was therefore concluded to be 320 mg/kg BW/day. However, the study could not be retrieved and references to this publication can be found in the assessments published by the National Research Council (1984, 2007) and a CIR assessment (CIR, 1983b). Some reproductive and developmental studies are also available for EA. In 1997, Hellwig and Liberacki fed pregnant Wister rats by gavage, water solutions containing 0, 40, 120 or 450 mg/kg BW/day of ethanolamine on days 6 to 15 of gestation. A total of 40 rats were allocated to each group. The NOAEL was reported as 120 mg/kg BW/day based on the lack of adverse events in this group. Material toxicity was observed at the highest dose group (decreased feed consumption, decreased body weight) with no associated developmental toxicity. Another reproductive and developmental toxicity study in pregnant rats by Mankes (1986) reported adverse effects in the progeny in all doses tested, although material toxicity was not observed. The animals in the study were given 0, 50, 300 or 500 mg/kg BW/day of EA by gavage from day 6 to 15 of gestation. A non-significant increase in the number of progeny with malformations was observed at all levels, but foetal lethality was only observed at the highest tested dose. Overall, male pups were significantly more affected by adverse events than female pups. Regardless of sex, pups contiguous to male siblings were reported to be more likely to experience adverse events than those contiguous to female siblings. The results of the study should, however, be interpreted with caution as the researchers did not follow the standard guidance for reproductive and developmental toxicity studies nor the regular methodologies for effect measurement or the litter as the statistical unit. Consequently, the study by Hellwig and Liberacki (1997) gives a more robust overview of the toxicity of EA.

In a study in pregnant mice (n=31), the animals were given 0 or 850 mg/kg BW/day of EA by gavage on day 6 to 15 of gestation (CIR, 1983a). Compared to the control, the treatment group showed increased mortality (16%) among other adverse events such as reduced number of viable litters, reduced survival rate of pups, decreased birth weight and decreased overall weight gain.

Additionally, different studies have also been conducted with EA hydrochloride. The production of hydrochloride salts of cosmetic and pharmaceutical ingredients is common in substances with limited solubility in water. EA hydrochloride is expected to be dissociated in the presence of water. An unpublished reproductive and development toxicological study in rats was cited in a CIR (Fiume et al., 2015). The study, sponsored by BASF in 2009, allocated 25 male and 25 females Wistar rats (F0 parental generation) to groups given a diet containing 0, 100, 300, or 1,000 mg/kg BW/day of ethanolamine hydrochloride for at least 75 days prior to mating. The same design was kept for the first generation of pups (F1) who continued receiving the same doses of ethanolamine hydrochloride. The study finished at the weaning of the second generation of pups (F2). The NOAEL was concluded at 300 mg/kg BW/day as adverse events on the gonads and fertility were observed at 1,000 mg/kg BW/day, although no developmental toxicity was observed in F1 or F2.

Based on the literature, the administration of EA is generally deemed safe even at high doses. However, there is evidence to suggest a potential for reproductive and systemic toxicity at elevated concentrations. Specific studies reported show a NOAEL of 300 mg/kg BW/day for repeated dose toxicity, while acute toxicity studies suggest relatively low acute toxicity with LD50. Based on the NOAEL of 300 mg/kg BW/day, and ADI of 0.3 mg/kg BW/day was calculated by applying a safety factor of 100 to account of intra- and inter-species variation and a further x10 to account for potential reproductive toxicity. Further information can be found in Appendix 21.

Figure 4 below shows the predictive toxicological profile obtained with Lazar Toxicology Prediction. The range of the results obtained with this program support the NOAEL obtained in the reproductive and system toxicity assay.

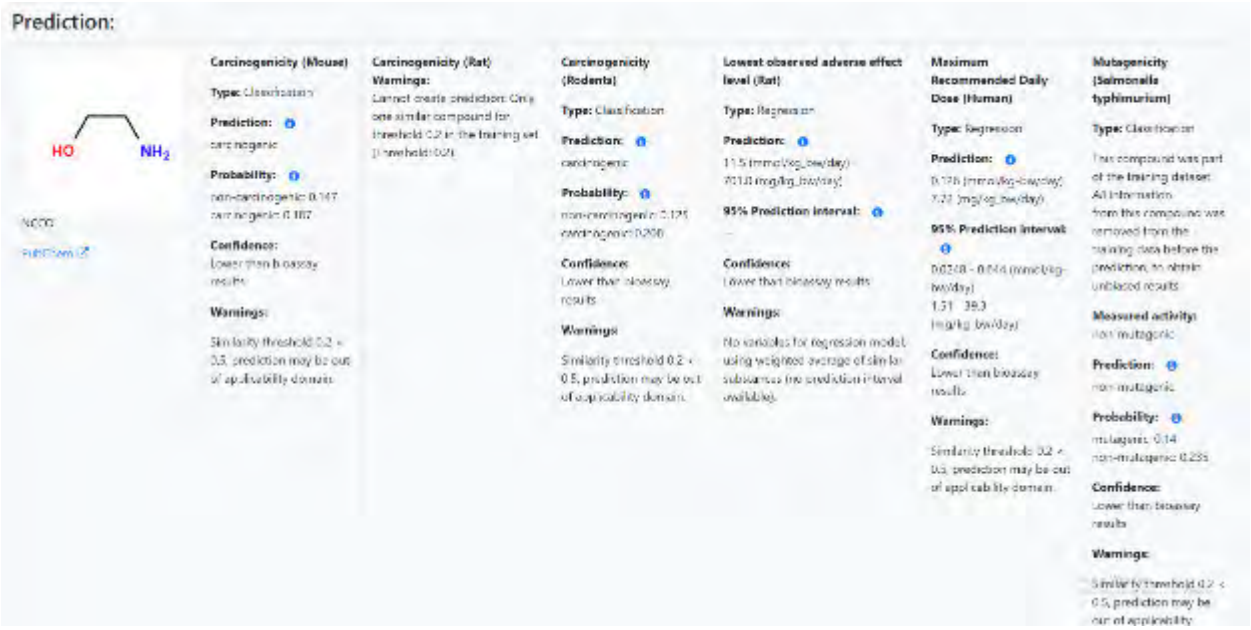

Figure 4. Lazar's prediction of ethanolamine's toxicological profile (February 2024)

Additionally, ToxTree was used to determine EA's Cramer class. The program classified the compound as Class I (low concern) based on the structure and the fact that it is an essential body constituent. Components in Cramer Class I are considered to have low probability of adverse health events if estimated intake is below 30 µg/kg BW/day.

Analytical testing was performed to evaluate the presence of EA in cell cultured duck (Confidential Appendix 8). The analytical data from five independent batches of cell cultured duck show that the levels of EA ranged between 3.0 and 13.3 µg/g (average: 6.94 µg/g). The estimated exposure to EA derived from cell cultured duck can be found in Table 45. The Margin of Exposure (MOE) (calculated by dividing the ADI by the estimated intake) is also presented in same table. The MOE is determined as 295.54 and 147.77 (mean and 95th percentile, respectively). Consequently, the exposure to EA derived from cell cultured duck can be considered safe.

C.3. Cell Metabolism and Toxins

Duck has been extensively consumed in many regions and cultures without any reported adverse events related to the intrinsic presence of toxins or other compounds that could pose a threat to human health. Hence, the genome of the cells can be assumed to be free from genes coding for these toxic compounds. The continuous cultivation of cells *in vitro* can cause epigenetic drifts such as a hyper- or hypomethylation of concrete sites, but cytogenetic changes may also occur during passaging. Consequently, small changes in genotype and phenotype may be possible during the cell line development process as the cells are repeatedly expanded. This is a naturally occurring process that also frequently happens *in vivo,* although *in vitro* cultures lack some of the biological mechanisms to prevent, repair and correct these alterations. However, these small changes in the genetic sequence are not considered harmful and are most of the time neutral. Any natural genetic changes that may occur during the cell line development are not considered harmful and cannot be realistically expected to result in the expression of substances of toxicological concern inside or outside the cellular space. The production of new or known toxins by aleatory, non-directed mutations in the genome of the cells cannot be realistically expected in duck cells in a controlled process. Very few species of wild birds are known to be poisonous to humans. However, the toxicity is linked to the bioaccumulation of toxins from the diet rather than to the production of the toxin within their organism *per se* (Yeung et al., 2022). Even if some mutations prompt changes in metabolic pathways leading to the build-up of toxic secondary metabolites in the cells, cells would either need to be resistant to their presence or these would need to be produced in concentrations that would not have an impact on cell growth and viability.

Moreover, any changes in the genetic expression of growth factors that may induce an under- or overexpression of certain proteins would likely translate into abnormalities in the PDT, growth rate and

phenotype. These changes would be noticeable after a few passages since these parameters are closely monitored in the routine cell culture QC tests.

The production of biogenic amines (BA) is, however, known to occur as part of the normal cellular activity and cell cultured duck was analysed for the presence of these compounds. BAs were analysed in five batches of cell cultured duck (Confidential Appendix 8). BAs can be commonly found in foods susceptible to rapid microbial degradation (such as fish) and partially or entirely fermented products (e.g., sausages, cheese), among others. Their presence in food results from exogenous enzyme activity or the microbial decarboxylation of amino acids during controlled or spontaneous fermentation, processing and storage (Durak-Dados et al., 2020). Certain BAs play a crucial role in cell maintenance, proliferation and differentiation, such as putrescine, spermidine and spermine which are widely present in eukaryotic and prokaryotic cells (Löser, 2000, Michael, 2016). However, high amounts of BA have been related to multiple health issues in the literature, although there is scarce information to derive a solid safety threshold in most cases. As Löser and others demonstrated, although some cells are capable of producing these compounds, exogenous BAs play a key role in cell metabolism. Precisely because of their ubiquitous nature and importance in key cell functions, BAs are present in numerous food products, especially in fermented products. In high amounts, BAs are undesirable substances, and their toxicity is well-established and reported in the literature. Of the BAs, putrescine and cadaverine are considered to be the least toxic, but research has stressed their potential to increase the toxicity of other BAs such as histamine or tyramine. It has been hypothesized that cadaverine and putrescine, in inadequate amounts, have the capacity to inhibit the enzymes responsible for metabolizing BAs (especially diamino oxidase or DAO), hence contributing to the absorption of histamine into the bloodstream and build-up in the cells (Sánchez-Pérez et al., 2022, Özogul and Özogul, 2020). Most of these effects have been studied *in vitro* and in rodents. The human data, however, show contradictory results. Researchers have suggested that the overall health and physiological status is an important factor to be considered. Individuals with histamine intolerance, reported allergies, gastrointestinal problems and other health issues may be at higher risk because of various factors linked to a lower DAO activity (Özogul and Özogul, 2020). However, no limits have been established for the and the levels of putrescine and cadaverine have yet not been legislated in the US or EU. Fermented products have been reported to have the highest putrescine concentrations (Özogul and Özogul, 2020, Durak-Dados et al., 2020). The concentrations of putrescine in harvested cell cultured duck range between 22.0 and 32.6 mg/kg, which are low compared to the natural concentration in other food commodities such as canned crab (122 mg/kg), matured cheddar (653 mg/kg), green pepper (54.7 – 90 mg/kg), orange (117 – 137 mg/kg) and grapefruit juice (98.6 mg/kg). Consequently, the amount of putrescine in cell cultured duck is safe and no adverse effects are expected. Spermidine and spermine concentrations ranged between 127-191 mg/kg and 111-171 mg/kg, respectively, for the harvested cell cultured duck (83.9% moisture). Muñoz-Esparza et al. (2019) reported that cereals, vegetables, fruits, nuts, oilseeds and legumes are the highest in spermidine and spermine. The concentrations of spermidine and spermine can range from 1 to 1,425 mg/kg and 0 to 722 mg/kg, respectively, depending on the food commodity. The concentrations in cell cultured duck are well within these ranges and consequently considered safe. Cadaverine and histamine could not be detected in any of the three batches analysed (below the analytical limit, < 1 mg/kg).

As for other toxic metabolites, it should be noted that Gourmey's cell line has not been genetically modified for genetically engineered and, consequently, no new genes that may code for harmful compounds have been introduced.

C.4. Toxicity

Gourmey's cell cultured duck is produced in a way that does not introduce additional new hazards or toxicological risks.

Animal cell cultivation techniques have been widely used in the pharmaceutical field. While culturing animal cells for human food consumption is a novel process, the cultivation of bacteria and yeast for producing enzymes, nutrient-rich biomass and other food ingredients is a process extensively used in the food industry. The safety of fermentation-derived foods is heavily dependent on the characteristics of the production strain (mainly: its ability to produce toxins, virulence factors, or molecules of concern, antibiotic resistance), the fermentation medium, the production process and the composition of the final product. Following this logic, it is concluded that:

1. Duck and, therefore, duck cells, have been traditionally and historically consumed in many parts of the world. There are no known cases of toxicity derived from the consumption of duck meat or derived products where these events were not caused by poor hygienic handling or contamination with ubiquitous food contaminants. Duck species do not contain any poisonous or venomous species. Therefore, the cells themselves are not toxic.
2. The composition of the Novel Food has been analysed in five different batches and protein has been identified as the major fraction present in cell cultured duck. No new constituents are expected to be present in the cells as these are derived from duck, which has traditionally consumed in Australia/New Zealand.
3. Cell cultured duck have been characterised and tested for the presence of media residues, food contaminants and biogenic amines. The results from five independent batches show that these are well within safe limits.
4. The toxicological profiles of media residues have been established through extensive literature reviews and no toxicological risks have been identified.
5. Cells have been fully characterised and are stable. The results presented throughout this dossier support genotypic and phenotypic stability.
6. The cell culture media used to produce the cell cultured duck is chemically defined and composed of food-safe components that do not introduce toxicological risks.

The production of new or known toxins by aleatory, non-directed mutations in the genome of the cells cannot be realistically expected in duck cells produced in a controlled process. Very few species of wild birds are known to be poisonous to humans. However, reported toxicity is linked to the bioaccumulation of toxins from the diet rather than to the production of the toxin within their organism *per se* (Yeung et al., 2022). Even if some mutations prompt changes in metabolic pathways leading to the build-up of toxic secondary metabolites in the cells, cells would either need to be resistant to their presence or these would need to be produced in concentrations that would not have an impact on cell growth and viability.

Moreover, any changes in the genetic expression of growth factors that may induce an under- or overexpression of certain proteins would likely translate into abnormalities in the population doubling time, growth rate, and phenotype. These changes would be noticeable after a few passages since these parameters are closely monitored in the routine cell culture tests. As previously discussed, the monitoring of growth stability is achieved by analysing the viable cells density, cell viability, and the doubling time to assess the cell proliferation quality. These parameters are checked routinely during production. Phenotype by microscopy can assess if the cells change their morphology throughout the production (e.g. aggregates, single cells, or differentiated cells).

An assessment of the cell cultivated duck biomass transcriptome was carried out in order to evaluate whether genes for toxic proteins are activated in the finished product. Protein sequences inferred from the most abundant gene transcripts identified in the finished biomass product, were compared with annotated protein sequences contained in UniProtKB followed by further analysis to determine their relevance.

In brief, the basis of the comparison was a standard approach involving detection of possible homology between transcript sequences and the toxin protein sequences in UniProtKB. Inclusive criteria were applied to avoid missing matches of possible relevance to toxicity. Homology between proteins of similar function is common, but conversely it is common for homologous proteins to have different functions, lacking the particular attributes necessary for a specific characteristic, such as toxin activity.

Many proteins have a modular nature, consisting of multiple domains (sequence/structure units) which can have very different functions or an essentially purely structural role. Therefore, analysis of homology between two proteins should include assessment of the locations of the matching segments, and their relevance to toxin function.

In total, 20 genes in the duck cells exhibited similarity with one or more toxin sequences at a level sufficient to warrant further examination. After detailed analysis, the similarities involved widespread protein domains, common to the toxins and a wide variety of functional protein types, and there was no evidence that the features specific to the toxin activity are present in the duck cell sequences. Consequently, many proteins in

food species and also in humans would be expected to make similar sequence matches with the domains in these toxin proteins.

The likely nature of the 20 genes was also investigated, by comparing with the annotated duck genome sequences available and with other avian reference sequences. In all cases, the gene descriptions were consistent with the presence of the protein domain types involved in the toxin-protein alignments.

In summary, the transcripts found in the finished biomass cells could be identified as expected as duck proteins, and no similarities to toxin database sequences suggested concerns regarding toxicity.

Based on the transcriptomic analysis, it is highly unlikely that the cells would produce toxins. This is consistent with the expectation that the production of new or known toxins by aleatory, non-directed mutations in the genome of the cells cannot be realistically expected in duck cells in a controlled process. Moreover, phenotypic characteristics are monitored during production and any changes in the performance of the cells would be identified and investigated. Cells in culture would be highly vulnerable to toxins, even if they were produced by the cells, impacting performance indicators. If abnormal growth performance was identified, a full investigation would be initiated to understand the cause. As it is highly unlikely that the cells would produce toxins, a transcriptomic analysis would only be performed if the cause could not be attributed to other more probable causes such as excessive lactate production or microbial contamination.

The report for this assessment along with the raw data is presented in Confidential Appendices 23a-c.

C.5. Assessment of allergenicity

To assess the allergenic potential of cell cultured duck, a weight of evidence (WoE) approach has been followed whereby a literature search and bioinformatic search was performed to identify known allergens in duck meat and eggs. Secondly, a transcriptomic analysis of cells from the WCB and cells from the end of production was performed, from which identified sequences were compared to allergen databases, and batches of the cell cultured duck were tested for egg allergen residues.

C.5.1. Assessment of duck proteins

The allergenicity risk assessment of duck meat protein and duck egg proteins is based on a comprehensive literature search, a review of publicly available allergen databases, and the analysis of egg proteins in cell cultured duck via ELISA (Confidential Appendix 8).

C.5.1.1. Allergenicity of duck meat

The search of published literature indicates that allergic reactions to avian meat are rare and that noted incidents of allergenicity have been mostly associated with chicken meat. Allergy to duck meat is uncommon and there is a lack of information found on the specific allergenicity of duck species (*Anas platyrhynchos*) in peer-reviewed journals, academic publications, and research repositories online. There are also no known international food allergen labelling regulations for duck.

Prevalence of allergies to poultry meat is discussed in (Hemmer et al., 2016) and it is noted that it is relatively rare. The study also suggested that the exact prevalence of poultry meat allergy is not well established, but it may presumably be similar to that of red meat. There is no close relationship between allergy to poultry meat and allergy of red meat. The study differentiates between primary poultry meat allergy and a secondary form associated with bird-egg syndrome. It is noted that primary poultry meat allergy stands for a true type 1 food allergy without a causal relationship to having allergies to eggs or bird feathers and that specific studies on the prevalence of genuine poultry meat allergy do not exist. Most cases are related to bird-egg syndrome which is characterized by an initial respiratory sensitization to bird antigens (e.g., feathers) that is followed by a secondary sensitization to egg or meat proteins. Alpha-livetin or chicken serum albumin (Gal d 5) (also found in egg yolk) has been reported as the main cross-reactive allergen in bird-egg syndrome, but it is rarely involved in primary allergic reactions to meat. Kelso et al. (1999b) investigated these cross-reactivity in different patients with allergy to poultry meat using immunoblotting assays that revealed IgE-binding protein bands at 8 kDa and 16 kDa in duck meat. The proteins, however, were not identified in the study. Other case

studies with patients allergic to poultry meat have been identified by Hemmer et al. (2016). They concluded that proteins involved in allergic reactions to poultry meat were low molecular weight proteins in the range of 5 and 25 kDa approximately. Among the identified proteins, the authors highlighted α-parvalbumin and myosin light chain 1. G as the main allergens in poultry meat. Klug et al. (2020) identified Gal d 7 as another major allergen in chicken meat which the authors also believed to be relevant in duck, turkey and goose. In fact, during the last decade, other proteins have also been reported as potential allergens in chicken meat, including haemoglobin, enolase (Gal d 9), aldolase (Gal d 10), α-actin and parvalbumin, but it is unclear how relevant these are to duck meat allergy. However, it should be noted that clinical cases prevail over in vitro studies as IgE-binding does not always translate into an allergic reaction *in vivo*. The knowledge on allergenicity poultry proteins other than egg still needs to be developed, hence no appropriate tests are commercially available yet.

However, it should be noted that egg or poultry meat allergy are typically independent conditions. A study evaluating two patients who had specific allergies to turkey and chicken meat, but no other food allergies was conducted on food allergies to poultry meat. Positive reactions for both chicken and turkey were confirmed through skin prick tests and specific IgE determination. Furthermore, there were documented instances of cross-reactivities to duck and goose meat. Immunoblot inhibition experiments confirmed the cross-reactivity of allergens found in chicken and turkey meat, suggesting the presence of shared allergenic components among various poultry meats, including duck. This research highlights that food allergy to poultry meat is a distinct and separate condition from egg allergies, as the patients were able to consume eggs and egg products without any adverse reactions (Cahen et al., 1998).

In a study conducted by González-Mancebo et al. (2011), a unique case of IgE-mediated allergy to chicken meat without egg protein sensitization was examined. The patient, who experienced allergic reactions shortly after consuming chicken, showed tolerance towards turkey but had not consumed other poultry meats. Skin prick tests with various poultry meats, including duck, were conducted. The study's key findings involved IgE immunoblotting assays, which revealed the patient's reactivity to specific protein bands in boiled duck extract. Notably, these bands showed complete inhibition of IgE-binding when preincubated with boiled chicken extract, suggesting cross-reactivity between chicken and duck proteins. The allergens identified in duck meat were α-parvalbumin and myosin light chain 1 (MLC), proteins also present in chicken and turkey meats. This investigation highlights the potential allergenicity of duck proteins and underscores the complexity of cross-reactivity among different poultry meats.

As part of the allergenicity assessment of duck meat, in addition to scientific literature search, multiple databases were used to search for known duck allergens. The databases used included the allergen database provided by the Food Allergy Research and Resource Program at the University of Nebraska (FARRP) (www.AllergenOnline.org), the Allergen Nomenclature database provided by the WHO/IUIS Allergen Nomenclature Sub-Committee (www.allergen.org), the Allergome database of allergenic molecules, https://www.allergome.org/, the Protein family (Pfam) database, the AllFam database of allergen families (https://www.meduniwien.ac.at/allfam/search.php), the Structural Database of Allergenic Proteins (SDAP), and the SWISS-PROT database.. All the databases, with the exception of the Allergome database, did not yield any results specifically associated with the allergenicity of *Anas platyrhynchos* or duck These databases did provide results for allergens associated with chicken (*Gallus gallus*) and eggs associated various avian species.

A review of the Allergome database of allergenic molecules did yield results for *Anas platyrhynchos* as Ana p [Meat] and Ana p 1-5. Upon further investigation, the referenced studies for Ana p [Mea] in the Allergome database indexed as immunochemistry and allergenicity references refer to egg-bird syndrome in childhood (Silva et al., 2009) and a clinical and immunological study of allergy to hen's egg white (Langeland, 1983). The other results listed for duck, and specifically *Anas platyrhynchos*, refer to molecules associated with egg allergies such as conalbumin, ovotransferrin, albumins, ovomucoids, and lysozyme.

The potential for duck allergens in products made from Gourmey cultivated duck to be overexpressed relative to conventionally sourced duck meat due to genetic drift is considered as not reasonably likely to occur. As discussed in Section B.4.2.2.2., cells taken from the WCB are stable during production. From vial thaw to the end of production, the cells maintained their pluripotency, the same number of chromosomes (karyotyping), normal growth characteristics and the ability to form EB which supports the stability of the cells in the semi-

continuous process. As the cells are phenotypically and genetically stable throughout the process, it is not likely that there is an increased potential for duck allergens in Gourmey cultivated duck as compared to conventionally sourced duck meat.

C.5.1.2. Duck Eggs

Allergic reactions to egg proteins have also been described in the literature, but mostly for chicken eggs. In fact, “eggs” are one of the major allergens of mandatory declaration under the Food Standards Code. As previously mentioned, extensive work has been done to understand, describe and establish the prevalence of allergenicity to chicken eggs, but little research is available on allergenicity to eggs from other bird species. Most cases of allergic reactions to duck eggs have been cases of cross-reaction in patients with known allergy to chicken eggs (Cahen et al., 1998, Langeland, 1983). Allergic reactions to duck or other bird eggs are extremely rare when these are not accompanied by allergy to chicken eggs (Añíbarro et al., 2000, Cahen et al., 1998, Alcántara Villar et al., 2019, Hemmer et al., 2016, Moghtaderi et al., 2020).

In a study from Kaminski (1962) investigated the cross-reactivity and potential allergenicity of ovalbumin from hens and ducks. Langeland (1983) revealed that the process of enzymatic digestion of duck ovalbumin yields precipitating fragments that are comparable to those obtained from hen ovalbumin. This suggests the likelihood of cross-reactivity and allergic responses between the two. The study also found that antibodies in anti-ovalbumin serum exhibit reactivity towards both the degraded fragments of ovalbumin and the intact heterologous ovalbumin, indicating a wide-ranging antibody response. Additionally, cross-reactions were observed between corresponding fragments of hen and duck ovalbumins, highlighting their immunological relationship and potential for allergenic cross-reactivity (Kaminski, 1962). The study highlights that the immunological reactions to proteins found in egg white cannot be relied upon to establish dietary limitations for individuals with egg allergies, as the allergenic characteristics of these proteins can be modified after boiling and digesting. It is pointed out that comprehensive clinical trials are necessary to have a complete understanding of the allergenic properties of egg whites derived from various avian species and their appropriateness in the dietary regimens of patients with allergies.

Anet et al. (1985) conducted a comparative study to evaluate the allergenicity of egg whites from different avian species, namely hen and duck eggs. The study revealed a notable level of cross-reactivity. The study employed serum samples from individuals with egg allergies to assess the allergic reaction. Roughly 50% of the examined cases exhibited significant cross-reactivity between hen and duck egg whites, indicating noteworthy similarities in their allergenic proteins. This finding emphasizes the potential genetic similarity in allergens found in both hen and duck egg whites and increases awareness of the potential for distinct sensitization to these proteins. Nevertheless, the study did not provide specific information regarding the precise doses and quantification of these effects (Anet et al., 1985).

Another study reported a paediatric patient with no previous chicken egg allergy having an IgE-mediated allergic reaction to uncooked quail egg, as verified by positive prick tests and specific serum IgE. Remarkably, the patient had previously ingested boiling quail eggs without experiencing any problems, indicating that the 75 kD protein known as ovotransferrin in quail egg white is probably sensitive to heat and loses its ability to trigger an immune response when boiled. The presence of this protein was not seen in eggs from chickens, ducks, or geese. The results suggest that although chicken eggs are a frequently encountered allergen, the patient's sensitivity is exclusive to quail eggs, without any cross-reactivity to chicken eggs. This case highlights the variation in the ability of bird egg proteins to cause allergies and the possibility of developing tolerance to eggs from some species even if one is allergic to eggs from other species. This behaviour corresponds to the documented bird-egg syndrome, in which the initial sensitivity of the respiratory system to bird proteins might result in subsequent intolerance to eggs. Nevertheless, the individual participating in the study exhibits an unusual manifestation, characterized by a primary sensitivity to egg protein without any prior respiratory symptoms (Caro Contreras et al., 2008).

In a study by Fernández Cortés et al. (2013), immunochemical responses of egg whites in various bird orders were investigated. The study revealed that ducks and geese have egg whites that are highly comparable to each other. Consequently, those who are sensitive to hen's egg white may experience allergic reactions when exposed to the egg whites of ducks and geese. Immunoblotting indicates that lysozyme may be an allergen, with its antigenic determinant being specific to Anseriformes and absent in Galliformes such as

chickens and turkeys. The diversity in allergenicity observed in egg whites from different avian species implies that those allergic to hen's egg white may still be able to tolerate eggs from other birds, such as ducks or geese. This case is a 69-year-old man who is able to tolerate hen's egg but has an allergic reaction to duck egg white. It demonstrates the potential for a specific type of allergy, known as IgE-mediated allergy, to duck egg white (Fernández Cortés et al., 2013).

Alcántara Villar et al. (2019) published investigated the allergenicity of duck egg proteins in a study case with a 25-year-old woman who suffered an allergic reaction after ingesting fried duck egg. A prick test revealed that the patient was allergic to duck egg white but not to chicken egg white. The researchers prepared fresh yolk and white duck egg extracts and performed an SDS-PAGE (acrylamide concentration, 16%) to separate the proteins. An immunoblotting assay by enhanced chemiluminescence was performed on these duck extracts and on commercially available chicken egg extracts, and the protein's IgE-binding capacity was tested by adding the patient's serum to all samples. The results showed a band between 37 and 50 kDa corresponding to ovalbumin (43-45 kDa). The researchers concluded that this was likely to be the protein responsible for the allergic reaction in the patient. Ovalbumin was also reported as an allergen in a study case in 2000 (Añíbarro et al.). In another case in 2013 where researchers applied very similar methodology, the allergic reactions in the patient were associated with lysozyme (Fernández Cortés et al., 2013).

In an investigation by Micozzi et al. (2016), the focus was on the allergenic differences between hen's egg and eggs from other species, including duck. According to the study, hen's eggs are a common allergen worldwide, and the main proteins that cause allergies in egg whites are ovalbumin, ovotransferrin, lysozyme, and ovomucoid. Among patients who are already tolerant to hen's eggs, the incidence of allergy to duck eggs and other non-hen eggs, such as quail's egg, is comparatively less common. Even in cases where patients were allergic to quail's egg, they frequently managed to tolerate hen's egg despite the cross-reactivity between the proteins from hen's egg and quail's egg. The study emphasizes that there are differences between allergic reactions to duck eggs and hen's egg, and that tolerance to one does not always imply tolerance to the other.

Based on the findings above, it is possible to conclude that allergies to duck eggs are tightly related to ovalbumin and lysozyme proteins. These are also two of the four protein fractions linked to chicken egg allergies ovalbumin (Gal d 2) and lysozyme proteins (Gal d 4). Ovomucoid (Gal d 1) and ovotransferrin (Gal d 3) (also called conalbumin) are the other two protein fractions associated with chicken egg allergies. None of these two have been reported as potential allergens in duck, although the published literature and information available is insufficient to conclude whether these could also be involved in allergies to duck egg. In fact, cross-reactions to bird proteins are relatively common and well-described in the literature, hence it is worth considering these two fractions as potential allergens associated with duck. The US FDA provides guidance, https://www.fda.gov/food/food-labeling-nutrition/food-allergies, to support this approach. The guidance details that consumers with egg allergies that for the purpose of allergen labelling, FDA interprets "eggs" as eggs from chickens but that the proteins in chicken eggs are very similar to those found in eggs from ducks, geese, quail and other birds and that people with egg allergies consult their health care provider before consuming eggs from other animals.

ELISA tests for egg allergens are widely used by the food industry. Egg allergen cross-contamination or egg allergic reaction is not a hazard likely to occur in cell cultured duck. However, different batches of cell cultured duck were tested using a validated ELISA test to verify this conclusion. ELISA results from different batches (Confidential Appendix 8) show that egg allergens were not detected in the batches of cell cultured duck tested indicating that cross-contamination of the cells with egg allergens has not occurred and or, the cells are not expressing egg allergens.

C.5.1.3. Transcriptomic analysis (amino acid sequence homology)

The Codex Alimentarius (2009) has published a methodology for assessing the potential allergenicity of derived foods derived from biotechnology, which can also be used as a model for evaluating the allergenic nature of novel proteins intended for human consumption (EFSA Panel on Dietetic Products and Allergies, 2014, EFSA Panel on Dietetic Products et al., 2021).

Following the principles outlined in FAO/WHO (2009), duck cells from the WCB and at end of production were sent for transcriptomic analysis using RNA-seq which consists of the preparation of samples to extract gene transcripts (RNA molecules), from which complementary DNA sequences (cDNA) are generated and then sequenced using a high throughput sequencing platform (Illumina NovaSeq) (Confidential Appendix 20a – Transcriptomics Report). The short-read data were assembled into full-length transcripts and analysed with standard statistical techniques to determine which transcripts are significantly more, or less, abundant in one type of sample compared to another. The study also included samples of retail-sourced duck meat, to aid contextual interpretation of any biomass genes/proteins which were flagged for similarity to allergen proteins.

The encoded protein sequences of the significantly higher-abundance gene transcripts were inferred from the transcript nucleotide sequences and compared to known sequences of allergenic protein in the following databases:

- COMPARE 2024 (2nd Feb 2024) (van Ree et al., 2021)
- Allergen Online (FARRP allergen protein database) V22 (25th May 2023) (Goodman et al., 2016)
- UniProt 2024_03, subset annotated as allergen (keyword KW-0020) (29th May 2024) (The UniProt Consortium, 2022) databases containing sequences of allergen proteins.

The criteria outlined in the current Codex guidelines for potential cross-reactivity are proteins exhibiting more than 35% amino acid sequence identity over a span of 80 or more amino acids (80mer) may indicate IgE cross-reactivity which could potentially trigger allergic reactions in sensitised individuals. Using a sequence identity < 50% is thought to rarely lead to cross-reactivity with known allergens, with 70% identity over most of the sequence considered to be a more realistic threshold (Aalberse, 2000, Ladics et al., 2011). For this study, a 50% threshold was used along with the criterion of 80 amino acids.

Expectation score (*E-scores*) which measure the degree of relatedness among protein sequences can help to distinguish random alignments from those with potentially significant structural similarities. Low *E*-scores (1e-5 or less), indicate functional similarity and may suggest a biologically significant relationship for allergenicity or cross-reactivity. In contrast, high *E*-scores, approximately 1.0 or greater, are usually associated with alignments that lack biological relevance (Henikoff and Henikoff, 1992, Henikoff and Henikoff, 1996, Pearson, 2000, Pearson, 2014, Pearson, 2016).

Using these criteria, evaluation of the predicted protein sequences found 30 alignments that were 80 amino acids or longer with a sequence identity of >50%. All E-scores were below 1e-5 indicating biological relevance of the sequence identity. The 30 different allergen sequence accessions comprise of relatively few different types of allergens (five in total) and unsurprisingly include instances of similar sequences from more than one reference database. The five include a number of proteins that are ubiquitous in vertebrates and so would be expected to be present in duck cells.

One of the key challenges in performing and interpreting transcriptomic analysis is there is a high degree of false positive hits for allergenic proteins that are highly conserved amongst species through extensive evolution that not predictive of allergenic potential based on observed taxonomic cross-reactivity (Abdelmoteleb et al., 2021). The majority of allergenic proteins identified in cell cultured duck are common proteins with an essential physiological function that are highly conserved. The main proteins identified with allergenic potential are discussed below.

Aldolases

Aldolases from salmon (*Salmo salar,* Sal s, 3, ALDOC), yellowfin tuna (*Thunnus albacares,* ALDOC), iridescent shark catfish (*Pangasianodon hypophthalmus*, ALDOC) and chicken (*Gallus gallus,* Gal d 10) were identified.

Aldolases are essential enzymes that play a crucial role in catalysing a critical reaction in glycolysis and gluconeogenesis. This enzymatic activity is vital for breaking down glucose to produce energy and for synthesizing glucose from smaller molecules. Aldolases are ubiquitous in various food sources, including meats, fruits, and vegetables. In animals, they are abundant in muscle tissues and organs, such as liver, due to their critical role in energy metabolism (Munegumi, 2015). Aldolases are also expected to be present in

embryonic stem cells as they are necessary for supporting rapid cell growth and division, which require efficient energy production (Kajita et al., 2000).

Aldolases from various species can exhibit high sequence homology due to the conserved nature of those enzymes. Sequence homology refers to the similarity in amino acid sequences between proteins from different species. In transcriptomics analyses, aldolases from fish species, such as salmon (*h*), may appear due to their conserved sequences across species. This homology is a basis for their inclusion in allergen databases and consideration in allergenicity assessments (Codex Alimentarius, 2009).

Aldolases are enzymes which have been described as allergens of clinical relevance in fish such as codfish, salmon and tuna (Hajeb and Selamat, 2012, Kuehn et al., 2013, Sharp and Lopata, 2014, Tomm et al., 2013). One study identified IgE positivity of 83% to aldolase in patients allergic to chicken and fish. Among seven patients with isolated chicken meat allergy, 6/7 were positive to aldolase. The allergen was recorded as Gal d 10 for chicken aldolase (Kuehn et al., 2016).

From transcriptomic analysis of cell cultured duck, the starter cells (WCB), and duck breast meat, the transcript abundance data from the duck breast meat shows that the pertinent gene Gal d 10 is more abundant in at least three of those samples, compared to all the starter culture samples and several of the finished biomass samples.

Muscle cells, such as those in duck breast, are metabolically active and require a continuous supply of energy to maintain muscle tone, support growth, and facilitate movement. As a result, enzymes involved in energy production, such as aldolase, are expected to be expressed in higher quantities to meet these energy demands (Bottje et al., 2017). In contrast, embryonic stem cells (ESCs) are in a different metabolic state compared to differentiated muscle cells. The metabolic needs of stem cells are focused on maintaining pluripotency and supporting cell division rather than producing large amounts of ATP for immediate use (Zhou et al., 2012). As such, it would be expected to have lower expression levels of glycolytic enzymes, like aldolase, in cell cultured duck as compared to conventional duck breast.

Gal d 10 is not considered to be one of the main allergens found in eggs. Clinically relevant allergens found in eggs are classified and characterised as Gal d 1 to 6 (EFSA Panel on Dietetic Products and Allergies, 2014), as such, the presence of Gal d 10 in cell cultured duck does not present a food safety concern.

Collagen

Collagen alpha-1 from salmon was identified. Collagen is ubiquitous in the animal kingdom, constituting the primary structural component of connective tissues such as skin, tendons, ligaments, and bones. It is found as an extracellular matrix protein in animals, and it plays a crucial role in maintaining tissue integrity and supporting various biological functions. As collagen is widely present in various food sources, particularly in animal-derived products such as meat, fish, and poultry muscle tissues and connective tissues, it is a significant component of the human diet.

While collagen is essential for structural integrity, its allergenicity is relatively rare compared to other proteins and it is not considered a major food allergen. Fish collagen has been identified as a significant allergen for some individuals, particularly those with fish allergies (Kalic et al., 2020). Although fish collagen has been proposed to be an allergen based on IgE-binding studies and two clinical case reports (Sakaguchi et al., 2000, Sakaguchi et al., 1999, Hamada et al., 2001, Kuehn et al., 2009, Liu et al., 2012), data from two double-blind placebo-controlled food challenge studies in fish-allergic patients (André et al., 2003, Hansen et al., 2003) suggest that its clinical importance is very limited. Thus, whereas fish collagen is a sensitiser, its ability to trigger allergic reactions is uncertain, in contrast to mammalian collagen, which can cause severe allergic reactions (Fritsche et al., 2010, Land et al., 2013). There is no IgE cross-reactivity between fish and mammalian collagens (Hamada et al., 2001), and no cross-reactivity between fish and bovine or porcine gelatines has been demonstrated (Sakaguchi et al., 1999; Andre et al., 2003).

Collagen from different species can have similar amino acid sequences, leading to sequence homology. This homology is a basis for their inclusion in allergen databases. Relevant to food, collagen from salmon is present in the match list, but collagens from bird species are not among those in the allergen databases.

The presence of collagen in cell cultured duck does not present a food safety concern.

Cyclophilin/peptidyl prolyl isomerase

Cyclophilin/peptidyl prolyl isomerase comprised 11 of the allergens identified.

Cyclophilins, also known as peptidyl prolyl isomerases (PPIases), are a family of enzymes that play a critical role in protein folding and function. Cyclophilins are found in all eukaryotic organisms, making them common in animal tissues, and are present in a wide range of arthropod, fungal and plant species. They are also expected to be present in various animal-derived foods, including meat, fish, and poultry as the enzymes are inherent to the cellular processes in muscle, liver, and other organs (Mukhtar et al., 2019). Therefore, these enzymes would be expected to be expressed in duck tissue and cell cultured duck.

As embryonic stem cells are highly proliferative and require efficient protein folding mechanisms to support rapid growth, cyclophilins are expected to be present because of their role in protein folding and maintenance of cellular homeostasis (Davis et al., 2010).

Cyclophilins / PPIases are not classified as major food allergens and are not considered an allergen for cell cultured duck.

The sequence homology of cyclophilins with known allergens in other species, such as dust mites, is the basis for their inclusion in allergen databases. However, this homology does not necessarily translate to allergenicity when these proteins are ingested despite their inclusion in allergen databases. When ingested, cyclophilins are broken down by the digestive system into smaller peptides and amino acids, which reduces their allergenic potential. This process helps in reducing the risk of allergenic responses as the smaller fragments are less likely to be recognized by the immune system as allergens (Pali-Schöll et al., 2018).

As cyclophilins are ubiquitous essential enzymes in protein folding and cellular function of eukaryotic cells, they are expected to be present in food. While cyclophilins are listed in allergen databases due to their homology with proteins in allergenic species like dust mites, they are not considered to be of allergenic concern in the context of food.

Tubulin

Tubulin accounted for seven of the identified allergens.

Tubulin is a globular protein that is a key building block of microtubules, which are essential components of the cytoskeleton in all eukaryotic cells. Microtubules are involved in various cellular processes, including maintaining cell shape, enabling cell motility, intracellular transport, and cell division. Tubulin is found in all eukaryotic organisms, making it ubiquitous in animal tissues. This includes muscle tissues, organs, and other cellular structures where microtubules play a critical role (Turk et al., 2015, Sharma et al., 2010, Mykles et al., 2000, Findeisen et al., 2014). As the proteins are ubiquitous, they would be expected to be expressed in duck tissue and cell cultured duck.

Tubulin is expected to be present in undifferentiated embryonic stem cells, such as those from ducks. These cells are highly proliferative and require robust microtubule networks for their rapid cell cycles and structural integrity. In embryonic stem cells, a well-maintained cytoskeleton is crucial for sustaining the undifferentiated state of the cells and their ability to differentiate into various cell types (Howard and Hyman, 2003). Rapid proliferation requires robust and dynamic microtubule networks to support the continuous cycles of cell division. The ability of microtubules to quickly polymerize and depolymerize allows the cells to adapt and respond to the demands of rapid division and differentiation (Liu et al., 2013).

Tubulin proteins themselves are not classified as major food allergens and are not considered an allergen for cell cultured duck.

The sequence homology of tubulins with known allergens in other species, such as dust mites and other arthropods, is the basis for their inclusion in allergen databases. However, this homology does not necessarily translate to allergenicity when these proteins are ingested. The reported allergenicity of tubulin proteins are in the context of inhalant allergies from sources like dust mites that can trigger allergic reactions. Dust mites are a common allergen source, and tubulin is one of the proteins identified in dust mite extracts reported in allergen databases. A study identified α-tubulin (Der f 33) as a novel allergen from the house dust mite *Dermatophagoides farinae*, which can induce allergic responses in sensitized individuals (Wang et al., 2016).

It is important to note that the route of exposure significantly impacts the allergenic potential. Tubulin can be allergenic when inhaled as part of dust mite allergens because it interacts directly with the immune system in the respiratory tract (Grant et al., 1977). However, when ingested, tubulin does not have the same allergenic effect. The process of digestion in the gastrointestinal tract breaks down proteins into smaller peptides and amino acids, reducing their allergenicity. This is particularly relevant for food allergens, as digestive enzymes can significantly alter the protein structures that are responsible for allergenic reactions. Studies have shown that the stability of food allergens to digestion is an important factor in determining their allergenic potential (Fu et al., 2002).

As tubulin is a ubiquitous structural protein found in many species and known to have allergenic potential in only specific forms in particular contexts (e.g., inhalation), it is not considered to be a protein of allergenic concern in the context of food.

Protease

Proteases, also known as peptidases or proteinases, are enzymes that catalyse the breakdown of proteins into smaller peptides or amino acids. They play a crucial role in various biological processes, including digestion, immune response, and cellular regulation. Proteases are found in all living organisms, including animals, plants, fungi, and bacteria. As the enzymes are ubiquitous, their presence and applications in food are well documented. Proteases like rennet are used in cheese production to coagulate milk proteins and proteases used in baking can modify gluten proteins to enhance dough properties and improve bread quality.

Certain proteases from food sources can be allergens. For example, papain from papaya and bromelain from pineapple are known to sometimes cause allergic reactions. Giangrieco et al., (2023) examined 341 Italian patients with allergies to see how common sensitization and allergic reactions to these enzymes were. The researchers found that 39% had specific IgE antibodies for at least one of the seven cysteine proteases tested. IgE antibodies are immune system proteins that play a key role in allergic reactions. The study demonstrated cross-reactivity of certain proteases from different sources. While most patients showed allergies to mite proteases, very few had allergic reactions to enzymes from papaya, pineapple, and fig. The study suggests that allergies to these plant enzymes are more likely to occur due to airway or dermal exposure in occupational settings rather than from food consumption.

The sequence homology of certain proteases, with known airway and/or dermal allergic reactions upon exposure from certain species of arthropods is the basis for their inclusion in allergen databases.

As proteases are ubiquitous essential enzymes in eukaryotic cells, they are expected to be present in food. While specific proteases are listed in allergen databases due to their homology with proteins in allergenic arthropods, fungi, and certain fruit, the proteases expected in conventional duck or cell cultured duck are not considered to be of allergenic concern in the context of food.

Differences in expression levels

Variations in gene expression levels from a transcriptomic analysis between the starter cells from the WCB and cells at the end of production were observed, with relative abundance of genes being higher in the end of production cells compared to the starter cells. This difference can be explained as cells taken from the WCB were cryopreserved and in an arrested state compared to the harvested duck cells which were cultured in suspension, produced in larger quantities and were metabolically active. Gene expression levels can be influenced by variations in the culture environment, cell cycle, cell density, and shear stress. A recent study reported that RNA expression profiles were impacted by cryopreservation and change during proliferation

(Heumüller-Klug et al., 2023). Additionally, studies have shown that gene expression profiles in ESCs are influenced significantly by the culture environment, including the use of serum-based cultures which can provoke gene expression variability (Guo et al., 2016) and cell density at harvest can affect gene-expression patterns in stem cells (Kim et al., 2014). Shear stress, resulting from the movement of the culture medium, can also affect the gene expression profiles of ESCs. It has been observed that shear stress can modulate the expression of genes involved in cell signalling and differentiation (Schneider et al., 2020). These factors collectively impact cellular signalling pathways that can lead to differences in gene expression profiles overtime, while the cells themselves remain undifferentiated.

The potential for duck allergens in products made from cell cultured duck to be overexpressed relative to conventionally sourced duck meat due to genetic drift is considered as not reasonably likely to occur. As discussed in Section B.4**,** cells taken from the WCB are stable during production. From vial thaw to the end of production, the cells maintained their pluripotency, the same number of chromosomes (karyotyping), normal growth characteristics and the ability to form EB which supports the stability of the cells in the semi-continuous process. As the cells are phenotypically and genetically stable throughout the process, it is not likely that there is an increased potential for duck allergens in Gourmey cultivated duck as compared to conventionally sourced duck meat.

The weight-of-evidence indicates that primary duck meat allergy is rare and that there is not an increased potential for duck allergens in cultivated duck meat as compared to conventionally sourced duck meat. As referenced in Kelso et al., (1999a), people that are allergic to one bird meat, such as chicken, may be allergic to others, including game birds, due to possible cross-reacting allergens and therefore they may have to exercise caution even when eating bird meats they have not previously ingested (Kelso et al., 1999a). Even within allergies to poultry meat, such as chicken (*Gallus gallus)* they generally only lead to mild symptoms, despite several proteins having IgE-binding capacity. Moreover, most of the reported cases relate to patients with “bird-egg syndrome”, a sensitization to egg yolk and other bird allergens as a result of a previous sensitization to airborne bird proteins (e.g., feather proteins) rather than primary poultry meat allergy.

C.5.1.4. Protein analysis

ELISA tests for egg allergens are widely used by the food industry. Egg allergen cross-contamination or egg allergic reaction is not a hazard likely to occur in cell cultured duck. However, different batches of cell cultured duck were tested using a validated ELISA test to verify this conclusion. ELISA results from different batches (Confidential Appendix 8) show that egg allergens were not detected in the batches of cell cultured duck tested indicating that cross-contamination of the cells with egg allergens has not occurred and or, the cells are not expressing egg allergens.

C.5.1.5. Allergenicity to media components and ingredients

The different media used by Gourmey do not contain any allergens of mandatory declaration under the current FSANZ legislation. The media components are not expected to cause hypersensitivity reactions. The final products will be labelled in accordance with the allergen labelling requirements if these contain any allergens.

C.5.1.6. Allergenicity to recombinant proteins

As previously, the recombinant bovine insulin used in the production process is produced by *P. pastoris* (or *K. pastoris*). The certificate of analysis (Confidential Appendix 16) shows the amount of residual host protein present in the insulin is 1,072.5 ng/mg. Proteins from *P. pastoris* are not known for being allergenic as demonstrated by Reyes et. al (2021). Jin et al., (2018) investigated the potential allergenicity of 17 residual *Pichia pastoris* host proteins by a literature review and bioinformatic sequence comparisons to known allergens. The literature showed no evidence of allergenicity of these proteins. 11 proteins were concluded to have modest identity to matches to environmental allergens with 13 proteins matching proteins from toxic sources however, these matches were similar to proteins from commonly consumed fungi including baker’s yeast. As such, it is highly unlikely that the fraction of host strain protein presents any serious food allergen concern.

C.6. Safety assessment reports prepared by international agencies or other national government Agencies

Gourmey's cells have not been yet assessed by any international or national agencies.

In 2023, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) jointly published "Food Safety Aspects of Cell-Based Food which examines the production technologies, potential hazards, and regulatory frameworks associated with cell-based foods (FAO, 2023). It includes a literature synthesis on terminology, production processes, and global regulatory landscapes, featuring case studies from Israel, Qatar, and Singapore. The report also emphasizes the importance of focusing on materials, inputs, and equipment specific to cell-based food production to ensure safety.

The UK Food Standards Agency published a risk assessment on the Identification of hazards in meat products manufactured from cultured animal cells in 2023 (FSA, 2023). This report identifies potential hazards in cultured animal cell meat production to support future FSA risk assessments. While focused on meat, similar risks may apply to seafood. It scopes out this novel technology to understand and assess emerging risks.

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

Cell cultured duck will be used as a food ingredient for further processing in the manufacture of cultured foie gras and other cell cultured duck analogues such as cultured minced duck meat, cell cultured duck breast.

Cell cultured duck foie gras and products are intended to be an alternative to conventional foie gras and duck meat and, therefore, the same or similar consumption patterns as their conventional counterparts are expected. Foie gras can be consumed as a dish (e.g., pan-seared foie gras) or can be used as an ingredient in multiple dishes (e.g., cannelloni with foie gras). A piece of pan-seared foie gras is considered a serving size (40-80 g), whilst the average serving size for spreadable foie gras (pâté), and duck pâté is estimated to be 28 g to 60 g (based on the serving size for foie gras and various pâtés reported in the United States Department of Agriculture (USDA) FoodCentral Database). For duck products, the estimated serving size is based on that of conventional duck meat (137 g) as reported for 'duck, domesticated, meat only, raw' category in the USDA FoodData Central database. However, it should be noted that the serving size largely varies depending on the type of product and database. Similar serving sizes are assumed for cell cultured duck products.

Cell cultured duck will be combined with other suitable ingredients to produce different finished products. It is the intent of Gourmey to ensure final products made from cultivated duck and other approved food ingredients receive appropriate pathogen lethality using validated thermal or non-thermal processing.

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

The cell cultured duck will be added to the finished products at an inclusion rate of 5% to 80% depending on the application.

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

The proposed inclusion rate of cell cultured duck in the categories above is between 5 – 80% depending on the finished product. The estimated exposure to cell cultured duck was calculated using the Australian Health Survey: Nutrition First Results – Foods and Nutrients, 2011-2012 (ABS, 2015c). Since no specific food group for duck meat was listed in the survey outcome, the daily intake of the food groups "Other poultry", "Liver paste, pate and dishes" and "mixed dishes where poultry or feathered game is the major component" were considered to estimate the exposure of cell cultured duck. The estimation of the intake is considered to be an overestimation of the exposure to the cell cultured duck, as cell cultured duck is mainly considered to

alternative to duck meat and foie gras. The intake values per kg body weight (kg/BW) were obtained by dividing the daily intake by 87 kg and 72 kg based on the mean body weight of males and females respectively reported in the National Health Survey 2017-18 (ABS, 2018). Assuming the conservative case in which cell cultured duck will 100% replace "Other poultry", "Liver paste, pate and dishes" and "mixed dishes where poultry or feathered game is the major component" in the diet and the maximum inclusion rate of cell cultured duck at 80%, on average, 26.9 g/day of cell cultured duck would be consumed among the adult population (Table 18).

Table 18. Estimated mean daily intake by gender			
Estimated mean daily intake of other poultry, liver paste, pate and dishes and cell cultured duck in Australia (g/day) by gender			
Food category	**Males**	**Females**	**Average**
Other poultry	1.3	1.2	1.3
Liver paste, pate and dishes	0.1	0	0.05
Mixed dishes where poultry or feathered game is the major component	36.9	27.7	32.3
Cell cultured duck	30.6	23.1	26.9
Estimated mean daily intake of other poultry, liver paste, pate and dishes and cell cultured duck in Australia (mg/kg body weight/day) by gender			
Food category	**Males**	**Females**	**Average**
Other poultry	14.94	16.67	15.8
Liver paste, pate and dishes	1.15	0	0.6
Mixed dishes where poultry or feathered game is the major component	424.14	384.72	404.43
Cell cultured duck	352.18	321.1	336.64

The estimated exposure to cell cultured duck was also determined per age group using data derived from the same survey above (Table 19).

Table 19. Estimated mean daily intake among adults by age group									
Estimated mean daily intake of other poultry, liver paste, pate and dishes and cell cultured duck in Australia (g/day) among adults by age group (years)									
Food category	**2-3**	**4-8**	**9-13**	**14-18**	**19-30**	**31-50**	**51-70**	**71 and over**	**Average**
Other poultry*	12.7	13.3	21.0	17.8	33.8	29.3	23.8	23.0	27.5
Organ meats and offal, products and dishes	0	0	0.2	0	0.2	0	0.6	0.5	0.3
Mixed dishes where poultry or feathered game is the major component	16.3	22.2	34.7	38.6	44.5	36.7	25.2	15.4	29.2
Cell cultured duck	13.548	18.292	28.76	31.592	37.112	30.532	21.592	13.64	24.7
*Taken as a fraction of the major group poultry and feathered game									

From the above assumptions, males would consume more cell cultured duck (30.6 g/day; 352.18 mg/kg bw/day) compared to females (23.1 g/day; 321.1 mg/kg bw/day). Adults between the ages of 19-30 would be the highest consumers of cell cultured duck (27.2 g/day).

The 95% percentile intake data were estimated based on FDA guidelines (2006), whereby the 95th percentile is usually considered 4 times the average intake for the most commonly consumed foods. Since foie gras and duck meat are not frequently consumed compared to other poultry products, a pseudo-95th percentile has been estimated by doubling the average intake. Using the estimated daily intake data in Table 20 which presents a more conservative scenario on the consumption of cell cultured duck, an estimated 95th percentile intake for cell cultured duck is determined as 53.6 g/day and 673.28 mg/kg BW/day.

Table 20. Estimated daily intake of duck meat products and cell cultured duck in Australia				
Food category	g/day		mg/kg BW/day	
	Mean	95th percentile	Mean	95th percentile
Other poultry	1.3	2.6	15.8	31.6
Liver paste, pate and dishes	0.05	0.1	0.6	1.2
Mixed dishes where poultry or feathered game is the major component	32.3	64.6	404.43	808.86
Cell cultured duck	26.9	53.8	336.64	673.28

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

Since cell cultured duck products and foie gras are considered a category of its own within meat and derives, all products within this food group will be added the novel food within the proposed inclusion rate (5% to 80%).

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

There is no extensive history of consumption of food derived from cell culture including cell cultured duck.

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

Cell cultured duck "foie gras" and products are intended to be an alternative to conventional foie gras and duck meat and, therefore, the same consumption patterns as their conventional counterparts are expected. Foie gras can be consumed as a dish (e.g., pan-seared foie gras) or can be used as an ingredient in multiple dishes (e.g., cannelloni with foie gras). A piece of pan-seared foie gras is considered a serving size (40-80 g), whilst the average serving size for spreadable foie gras, and duck pâté is estimated to be 28 g to 60 g.

For duck products, the estimated serving size will be based on that of conventional duck meat.

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

There is currently no approval for cell cultured duck in any market. However, as mentioned in Section 4, cell culture-derived ingredients have been approved by several regulatory bodies including the US, Singapore and Israel.

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 novel food is unlikely to create any nutritional imbalance as the ingredient will be used in products that will be formulated in such a way that they recreate the nutritional characteristics of their conventional counterparts.

A comparison of the nutritional profile of cell cultured duck and conventional duck meat shows some similarities although they are not strictly equivalent (Table 21-Table 23). The protein content of cell cultured duck is lower than the average value for conventional duck breast although comparable to the value reported by FAO (2001). Conventional duck meat is also generally richer in fat as opposed to cell cultured duck but with similarly low levels of carbohydrates and ash. The moisture content of cell cultured duck is higher than the references of conventional duck breast obtained. Although comparing the nutritional profile of cell cultured

duck against conventional duck meat can provide useful context for the interpretation of the nutritional profile, there are inherent differences in metabolic pathways and cellular diversity between live animals and cell cultured duck.

Unlike conventional animal cells derived from whole organisms, cells derived from culture are produced under controlled conditions that do not fully replicate the complex biological processes and cellular interactions found in live animals. Cell cultivation focuses on controlled growth of specific cell types in a reproducible and scalable manner. Although the production methods and underlying biological context may differ, both cell cultured, and conventional duck are nutritionally safe, and cell cultured duck is not nutritionally disadvantageous in the context of a healthy and varied diet. However, both are nutritionally safe in the context of a healthy and varied diet.

Table 21 Proximate profile of Gourmey's cell cultured duck compared to conventional duck meat products

Nutritional Parameter	Gourmey's Cell cultured duck (g/100 g)	Conventional Duck Breast (g/100 g)[1]	Duck Meat (g/100 g)[2]	Offal liver ducks (g/100g)[2]	Duck, breast, lean, raw (g/100g)[3]
Energy value	57.4	340	291	136	91.5
Moisture	84.94	53.2	-	-	77.5
Ash	1.41	1.24	-	-	1.3
Protein	12.07	17.9	8.3	18.7	21.2 g
Carbohydrate	< 0.1	0.85	-	-	0
Total Fat	1.36	29.4	28.3	4.6	0.6

[1](ANSES-CIQUAL, 2020)
[2](FAO, 2001, ANSES-CIQUAL, 2020)
[3]Australian Food Composition Database – Release 2.0

Table 22. Vitamin and mineral profile of Gourmey's cell cultured duck compared to duck breast

Compositional parameters	Gourmey's cell cultured duck (g/100 g)	Duck, breast, lean, raw (g/100g)[3]
Sodium (g/100 g)	0.108	0.06
Calcium (mg/kg)	13.04	40
Iron (mg/kg)	134.85	32
Fluoride (mg/kg)	< 1	-[2]
Potassium (mg/kg)	3,640	2700
Phosphorus (mg/kg)	2,928	2300
Magnesium (mg/kg)	223	260
Manganese (mg/kg)	0.308	0.17
Zinc (mg/kg)	28.30	11
Vitamin A, retinol (µg/100 g)	<21	0
Vitamin B1 (mg/100 g)	0.2[3]	0.37
Vitamin B2, riboflavin (mg/100 g)	0.173	0.28
Vitamin B3, niacin (mg/100 g)	38.940	6.8
Vitamin B5 (as calcium pantothenate) (mg/100 g)	0.704	1.8
Vitamin B6, pyridoxine (mg/100 g)	0.289	0.21
Vitamin B8, biotin (µg/100 g)	41.92	-[2]

Table 22. Vitamin and mineral profile of Gourmey's cell cultured duck compared to duck breast		
Compositional parameters	**Gourmey's cell cultured duck (g/100 g)**	**Duck, breast, lean, raw (g/100g)[3]**
Vitamin B9, folate (µg/100 g)	92.10	0
Vitamin B12 (µg/100 g)	0.512	2
Vitamin C (mg/100g)	0.2[3]	0
Vitamin D3 (µg/100 g)	0.466	0
Vitamin E (mg/100 g)	< 0.08	0.9
Vitamin K1 (µg/100 g)	< 0.8	-[2]
Choline (mg/100g)	0.2[3]	-[2]
Molybdenum (mg/100g)	0.0001[3,4]	-[2]
Iodine (mg/100g)	0.0001[3,4]	0
Chromium (mg/100g)	0.001[3,4]	-[2]
Selenium (µg/100 g)	0.02	27

[1] Australian Food Composition Database – Release 2.0
[2] Value not available
[3]Parameter was not analysed in the final cell cultured duck biomass. The value shown is the input level of the substance present in the culture media A conservative approach of input level = output level has been used to obtain a value for the above comparison
[4] Taken from chemically-defined patent of formulation used in early isolation stage

Table 23. Fatty profile of Gourmey's cell cultured duck compared to duck breast		
Fatty acid	**Gourmey's Cell cultured duck (g/100 g)**	**Duck, breast, lean, raw[1]**
Omega 3 fatty acids	0.009	0.0136
Omega 6 fatty acids	0.002	-[2]
C4:0 butyric acid	0.001	0
C6:0 Caproic acid	0.001	0
C7:0 Enanthic acid	0.001	-[2]
C8:0 Caprylic acid	0.001	0
C9:0 Pelargonic acid	0.001	-[2]
C10:0 Capric acid	0.001	0
C11:0 Undecyclic acid	0.001	-[2]
C11:1 Undecylenic acid	0.001	-[2]

Table 23. Fatty profile of Gourmey's cell cultured duck compared to duck breast		
Fatty acid	**Gourmey's Cell cultured duck (g/100 g)**	**Duck, breast, lean, raw[1]**
C12:0 Lauric acid	0.003	0
C12:1 Lauroleic acid	0.001	-[2]
C13:0 Tridecyclic acid	0.001	-[2]
C13:1 Trydecylenic acid	0.001	-[2]
C14:0 Myristic acid	0.024	0
C14:1 (n-5c) Myristoleic acid	0.002	0
C14:1 (n-5t) Myristoleic acid	0.001	0
C15:0 Pentadecyclic acid	0.001	0
C15:1 (n-5c) Pentadecenoic acid	0.001	-[2]
C15:1 (n-5t) Pentadecanoic acid	0.001	-[2]
C16:0 Palmitic acid	0.330	0.12
C16:1 (n-7c) Palmitoleic acid	0.069	0.01
C17:0 Margaric ac.	0.001	0
C17:1 (n-7c) Heptadecenoic ac.	0.001	-[2]
C17:1 (n-7t) Heptadecenoic ac.	0.001	-[2]
C18:0 Stearic ac.	0.2	0.07
C18:1 (n-6c)	0.001	0.17
C18:1 (n-7c) Vaccenic ac.	0.002	-[2]
C18:1 (n-7c) trans Vaccenic ac.	0.001	-[2]
C18:1 (n-9c) Oleic ac.	0.593	-[2]
C18:1 (n-9t) + C18:1 (n-12t)	0.024	-[2]
C18:2 (9c, 11t) Conjugated linoleic ac.	0.001	-[2]
C18:2 (n-6c) linoleic ac. (LA) ω6	0.003	0.09
C18:2 (n-6c) linoleic ac.	0.001	0.09
C18:2 t2	0.001	-[2]
C18:3 (n-3) α-linoleic ac. (ALA) ω6	0.001	0
C18:3 (n-6) γ-linoleic ac. (GLA) ω6	0.001	0
C18:3 t3 (C18:3 t1+C18:3 t2)	0.001	-[2]
C18:4 (n-3) Moroctic Ac. ω3	0.010	0
C19:0 Nonadecylic ac.	0.004	-[2]
C19:1 (n-12t)	0.001	-[2]
C19:1 (n-9t)	0.001	-[2]
C20:0 Arachide ac.	0.005	-[2]
C20:1 (n-9c) Gondoic ac.	0.023	0
C20:1 (n-9t) + C18:2 (10t, 12c)+C20:1 (n-15c)	0.001	-[2]

Table 23. Fatty profile of Gourmey's cell cultured duck compared to duck breast		
Fatty acid	**Gourmey's Cell cultured duck (g/100 g)**	**Duck, breast, lean, raw[1]**
C20:2 (n-6c) Eicosodienoic ac.	0.001	0.00227
C20:3 (n-3c) Eicosatrienoic ac.	0.001	0
C20:4 (n-6c) Arachidonic ac. (AA) ω6	0.001	0.0482
C20:5 (n-3c) Eicosapentaenoic (EPA) ω3	0.001	0.00397
C21:0 Heneicosanoic ac.	0.001	-[2]
C22:0 Behenic ac.	0.001	0
C22:1 (n-11) cetoleic ac.	0.003	-[2]
C22:1 (n-9c) Erucic ac.	0.001	-[2]
C22:1 (n-9t) Brassidic ac.	0.005	-[2]
C22:2 (n-6c) Docosadienoic ac.	0.001	0
C22:3 (n-3c) + C22:4 (n-6c)	0.001	-[2]
C22:5 (n-3c) Docosapentaenoic ac. (DPA) ω3	0.001	0.0051
C22:5 (n-6c) Docosapentaenoic ac. ω6	0.001	-[2]
C22:6 (n-6c) Docosapentaenoic ac. ω6	0.001	-[2]
C24:0 Lignoceric ac.	0.001	0
C24:1 Nervonic ac.	0.005	-[2]

[1] Australian Food Composition Database – Release 2.0
[2] Not reported

E.1.1. Protein quality

As seen below in Table 24 cell cultured duck has an adequate essential amino acid profile observed from the analysis of five batches containing all the essential amino acids although in lower levels than conventional duck for certain amino acids.

Table 24 Comparison of amino acid profile of cell cultured duck with conventional duck							
Amino acid	**RB-00D1 (g/100 g)**	**RB-001C (g/100 g)**	**RB-001G (g/100 g)**	**RB-002B (g/100 g)**	**RB-002D (g/100 g)**	**Conventional duck (g/100 g)[1]**	**Duck, breast, lean flesh, raw (g/100g)[3]**
Glycine	0.622	0.580	0.567	0.543	0.345	0.808	0.841
Histidine	0.296	0.286	0.273	0.26	0.166	0.469	0.572
Aspartic acid	1.15	1.08	1.10	1.1	0.658	1.707	1.627
Glutamic acid	1.64	1.53	1.51	1.45	0.915	2.586	2.690
Proline	0.540	0.526	0.499	0.505	0.297	0.717	0.789
Hydroxyproline	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	-[2]	-[2]
Serine	0.561	0.541	0.512	0.518	0.309	0.722	0.809
Ornithine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-[2]	-
Phenylalanine	0.531	0.511	0.488	0.461	0.296	0.741	0.784

Table 24 Comparison of amino acid profile of cell cultured duck with conventional duck							
Amino acid	**RB-00D1 (g/100 g)**	**RB-001C (g/100 g)**	**RB-001G (g/100 g)**	**RB-002B (g/100 g)**	**RB-002D (g/100 g)**	**Conventional duck (g/100 g)[1]**	**Duck, breast, lean flesh, raw (g/100g)[3]**
Lysine	1.04	0.982	0.949	0.943	0.618	1.632	1.456
Methionine	0.262	0.280	0.273	0.241	0.144	0.225	0.496
Isoleucine	0.554	0.524	0.502	0.483	0.301	0.808	0.992
Leucine	1.03	0.985	0.935	0.883	0.563	1.546	1.476
Valine	0.677	0.638	0.614	0.584	0.367	0.921	0.966
Alanine	0.688	0.650	0.661	0.611	0.386	1.151	1.066
Threonine	0.546	0.519	0.503	0.491	0.295	0.865	0.972
Tyrosine	0.446	0.443	0.415	0.392	0.295	0.594	0.658
Arginine	0.876	0.846	0.816	0.786	0.490	1.108	1.163
Cystine+cysteine	0.152	0.156	0.152	0.241	0.094	0.166[4]	0.222
Tryptophan	0.154	0.152	0.148	0.169	0.096	-[2]	0.170[3]

[1]Values obtained from Zhang et al. (2023)
[2]Not reported
[3] Australian Food Composition Database – Release 2.0
[4]Value for only cysteine as cystine was not reported

Table 25 shows that levels of essential amino acids in mg/g of protein in cell cultured duck can be comparable to the levels present in chicken egg and beef. The average essential amino acid content obtained from the analysis of five batches of cell cultured duck was calculated in mg/g of the average protein content of cell cultured duck obtained from the proximate analysis of five batches (Table 13).

Table 25. Levels of essential amino acids per protein content			
Essential amino acid	**mg per g of protein**		
	Cell cultured duck	**Chicken egg[1]**	**Beef[1]**
Histidine	21.8	23	26
Phenylalanine plus tyrosine	70.6	94	74
Lysine	75.3	67	71
Methionine plus cystine	33.19	65	32
Isoleucine	39	50	35
Leucine	73	85	70
Valine	48	59	40
Threonine	39	48	38
Tryptophan	12	13	9

[1]Values obtained from USDA's FoodData Central

In terms on antinutrients, no known antinutrients are used in the culture media as listed in Appendix 5a, and in terms of the cells themselves, in general, anti-nutritional factors are associated with foods from plant origin (e.g. lectins and tannins) and anti-nutritional factors in cell-cultured duck are not expected.

Taken together, as part of a healthy and balanced diet, cell cultured duck can be reasonably expected to be nutritionally adequate for all population groups considering that cell cultured duck would not be the sole source of dietary protein or any other nutrient.

E.1.2. Exposure to nutrients

The exposure to the most relevant nutrients identified has been determined using the average of the nutrient level obtained from the analysis of five batches of harvested cell cultured duck (Table 26). In the sections below, the nutrient levels in cell cultured duck are compared to the current Nutrient Reference Values (NRV) for Australia and New Zealand published by the National Health and Medical Research Council (NHMRC) and the New Zealand Ministry of Health available at https://www.eatforhealth.gov.au/nutrient-reference-values/nutrients.

Table 26. Estimated exposure to vitamins and minerals derived from the consumption of cell cultured duck

Nutrient	Exposure	Cell cultured duck
P	Content (mg/kg)	2,928
	Mean (mg/day)	78.76
	95th percentile (mg/day)	157.53
Fe	Content (mg/kg)	138.85
	Mean (mg/day)	3.63
	95th percentile (mg/day)	7.25
K	Content (mg/kg)	3,640
	Mean (mg/day)	97.92
	95th percentile (mg/day)	195.83
Mg	Content (mg/kg)	222.3
	Mean (mg/day)	5.95
	95th percentile (mg/day)	11.91
Folate	Content (µg/100 g)	92.10
	Mean (µg/day)	22.77
	95th percentile (µg/day)	49.55
Zn	Content (mg/kg)	28.30
	Mean (mg/day)	0.76
	95th percentile (mg/day)	1.52
Mn	Content (mg/kg)	0.308
	Mean (mg/day)	0.0083
	95th percentile (mg/day)	0.0166

E.1.2.1. Phosphorus

Phosphorus is one the most abundant nutrients and plays a fundamental role in essential cell functions and physiological mechanisms such as homeostasis and kidney function. Although no specific restrictions apply to the overall population, those with kidney disease or impaired phosphorus metabolism should closely monitor the phosphorus in their diets as a high phosphorus daily intake can contribute to kidney overload, renal calcification and lead to other complications in this population group. The Recommended Daily Intake (RDI) of phosphorus for each population group is listed in Table 27. The Estimated Average Requirement (EAR) is also given. The Upper Level of Intake (UL) for phosphorus was estimated to be 3,000 mg/day for children (1-8 years old) and the elderly (>70 years old) and 4,000 mg/day for the children, adolescents and adults (9-70 years old).

Table 27. RDI, EAR and UL (mg/day) for phosphorus in population groups stratified by age			
Age	**RDI (mg/day)**	**EAR (mg/day)**	**UL (mg/day)**
≤6 months	100 (AI)	-	ND
7–12 months	275 (AI)	-	ND
1–3 years	460	380	3,000
4–8 years	500	405	3,000
9–13 years	1,250	1,055	4,000
14–18 years	1,250	1,055	4,000
19–70 years	1,000	580	4,000
>70 years	700	580	3,000
Notes: data obtained from (NHMRC, 2006d) AI (Adequate Intake); EAR (Estimated Average Requirement); RDI (Recommended Dietary Intake); UL (Upper Level of Intake); ND (Not Determined)			

Data on the usual daily intake of phosphorus has been taken from the Australian Health Survey: Usual Nutrient Intakes, 2011–12 reported as being among the most recent National Nutrition Surveys (NNs) in the Food Standards Australia New Zealand Application Handbook (FSANZ, 2024a). As presented in Table 28 this data reports an estimated daily mean intake and 95th percentile.The dietary intake data show that the intake of phosphorus is at least 2 times lower than the UL for all population groups.

Table 28. Estimated daily intake of phosphorus from foods in different population groups (mg/day)				
Age	**Mean**		**95th percentile**	
	Males	**Females**	**Males**	**Females**
Children 2-3y	1,113	985	1,455	1,303
Children 4-8y	1,178	1,044	1,535	1,372
Children 9-13y	1,449	1,251	2050	1,787
Adolescents 14-18y	1,621	1,230	2,246	1,771
Adults 19-30	1,716	1,249	2,360	1,785
Adults 31-50	1,707	1,295	2,357	1,843
Adults 51-70	1,574	1,297	2,195	1,843
Adults 71 and over	1,443	1,227	2,046	1,753
Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015b)				

Phosphorus is naturally present in many protein-rich foods including meat, fish and dairy products with a higher bioavailability than from plant sources (Biruete et al., 2023). It is also present in food additives as phosphate salts. As seen in Table 29, the potassium level in cell cultured duck (293 g/100g) is comparable to conventional meat products (230-271 mg/100g). Based on the maximum inclusion rate of cell cultured duck, the average and 95th exposure to phosphorus for the Australian population are estimated to be 78.76 mg/day and 157.53 mg/day, respectively. Considering this, cell cultured duck is not expected to negatively affect the consumer. The phosphorus daily intake from cell cultured duck is far below the UL of 4,000 mg/day and cell cultured duck is not expected to increase the exposure to this component significantly.

Table 29. Phosphorus concentration in different foods	
Food commodity	**Concentration (mg per 100 g)**
Cell cultured duck	293
Duck, breast, lean, raw	230
Egg, chicken, yolk, hard-boiled	402
Chicken, breast, lean flesh, raw	240
Chicken, breast, lean flesh, skin & fat, baked, no added fat	271
Beef, boneless dice or strips, lean, raw	240
Meat alternative products made from a fungus base	210
Meat alternative, legume and/or vegetable base	120
Cheese, cheddar, processed, regular fat	436
Yoghurt, natural, regular fat (3% fat)	156
Milk, cow, powder, regular fat, unfortified	810
Note: data obtained from the Australian Food Composition Database – Release 2.0	

E.1.2.2. Iron

Iron is an essential nutrient with key physiological functions in oxygen transportation throughout the body. Since iron is an essential constituent of haemoglobin, iron deficiency is frequently associated with anaemia. The average RDI for iron is between 8 and 13 mg/day for the general population excluding infants (>3 years) as seen in Table 30. The UL for iron is 20 mg/day for infants and young children (0-3 years old), 40 mg/day for children and young adolescents (4-13 years old) and 45 mg/day for the rest of the population. Since the bioavailability of iron is dependent on many factors, especially the type of iron (heme and non-heme forms), this factor was taken into consideration when estimating the population dietary requirements. Overall, only 18% of the dietary iron is expected to be absorbed (Institute of Medicine 2001).

Table 30. RDI, EAR and UL (mg/day) for iron in population groups stratified by age			
Age	**RDI (mg/day)**	**EAR (mg/day)**	**UL (mg/day)**
≤6 months	0.2 (AI)	-	20
7–12 months	11	7	20
1–3 years	9	4	20
4–8 years	10	4	40
9–13 years	8	6	40
14–18 years	13*	8	45
19–70 years	13*	6*	45
>70 years	8	6*	45

Notes: data obtained from (NHMRC, 2006b)
*Average of data for males and females
AI (Adequate Intake); EAR (Estimated Average Requirement); RDI (Recommended Dietary Intake); UL (Upper Level of Intake); ND (Not Determined)

The daily iron intake obtained from data in the Australian Health Survey is presented in Table 31. It can be seen that iron is consistently consumed at levels higher than the EAR and the RDI in some cases. The UL of iron is at least 2 times higher than the estimated daily intake for the whole population.

Table 31. Estimated daily intake of iron from foods in different population groups (mg/day)

Age	Mean		95th percentile	
	Males	Females	Males	Females
Children 2-3y	8	7	12	10
Children 4-8y	9	8	13	12
Children 9-13y	12	9	17	14
Adolescents 14-18y	13	9	19	14
Adults 19-30	13	9	19	14
Adults 31-50	13	9	19	14
Adults 51-70	12	10	18	15
Adults 71 and over	12	9	18	14

Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015b)

As seen in Table 32, cell cultured duck has a higher iron concentration (13.5 mg/100g) compared to conventional meat (0.26-3.2 mg/100g). Based on the maximum inclusion rate of cell cultured duck (80%), the average and 95th exposure to iron for the Australian population are estimated to be 3.63 mg/day and 7.25 mg/day, respectively. Considering this, cell cultured duck is not expected to negatively affect the consumer. Iron consumption levels are far from the UL of 45 mg/day set for the adult population and the consumption of cell cultured duck is not expected to increase the iron take beyond the UL. Moreover, a large proportion of the iron in cell cultured duck is expected to be non-heme as ferric citrate is the main source of iron in the food-safe culture media. The absorption of non-heme forms of iron is very low compared to that of heme iron.

Table 32. Iron concentration in different foods

Food commodity	Concentration (mg per 100 g)
Cell cultured duck	13.5
Duck, breast, lean, raw	3.2
Egg, chicken, yolk, hard-boiled	4.8
Chicken, breast, lean flesh, raw	0.26
Chicken, breast, lean flesh, skin & fat, baked, no added fat	0.57
Beef, boneless dice or strips, lean, raw	1.7
Meat alternative products made from a fungus base	0.4
Meat alternative, legume and/or vegetable base	1.8
Lamb, liver, grilled, no added fat	11

Lentil, red, hulled, dry	9.1
Bean, red kidney, dried	5.6
Note: data obtained from the Australian Food Composition Database – Release 2.0	

E.1.2.3. Potassium

Potassium is an essential nutrient with key physiological functions in muscle function, blood pressure among others. This mineral is widely present in food and hence deficiency is rare. No ULs have been set for potassium from dietary sources due to insufficient evidence and data however, Adequate Intake (AI) levels have been set for the different population groups as per Table 33.

Table 33. Adequate Intakes (AI) for potassium in population groups stratified by age

Age	Adequate Intake (AI) (mg/day)	
	Males	Females
0-6 months	400	400
7- 12 months	700	700
1-3 years	2,000	2,000
4-8 years	2,300	2,300
9-13 years	3,000	2,500
14-18 years	3,600	2,600
19-50 years	3,800	2,800
51+ years	3,800	2,800
Note: data obtained from (NHMRC, 2006e)		

The estimated daily potassium intake obtained from data in the Australian Health Survey. The average and 95th percentile for all age groups are presented below (Table 34).

Table 34. Estimated daily intake of potassium from foods in different population groups (mg/day)

Age	Mean		95th percentile	
	Males	Females	Males	Females
Children 2-3y	2,170	1,925	2,848	2,558
Children 4-8y	2,259	2,006	2,959	2,651
Children 9-13y	2,729	2,469	3,520	3,629
Adolescents 14-18y	2,909	2,396	3,710	3,561
Adults 19-30	3,187	2,449	4,023	3,606
Adults 31-50	3,289	2,618	4,149	3,816
Adults 51-70	3,172	2,717	4,016	3,932
Adults 71 and over	2,969	2,542	3,799	3,708
Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015b)				

As shown in Table 35, the levels of potassium in cell cultured duck (364 mg/100g) are comparable to those in meat commodities (340-390 mg/100g). Although the UL for potassium could not be determined, the concentration in cell cultured duck and conventional meat does not differ significantly to produce a nutritional imbalance or adverse effect. Moreover, potassium is a ubiquitous mineral in food and the lack of reported adverse events indicate that the safety margin is wider compared to other minerals.

Table 35. Potassium concentration in different foods	
Food commodity	**Concentration (mg per 100 g)**
Cell cultured duck	364
Duck, breast, lean, raw	380
Egg, chicken, yolk, hard-boiled	97
Chicken, breast, lean flesh, raw	390
Chicken, breast, lean flesh, skin & fat, baked, no added fat	303
Beef, boneless dice or strips, lean, raw	340
Meat alternative products made from a fungus base	120
Meat alternative, legume and/or vegetable base	340
Milk, cow, powder, skim	1,800
Potato, new, peeled, baked, no added fat	777
Bean, red kidney, dried	1,470
Note: data obtained from the Australian Food Composition Database – Release 2.0	

E.1.2.4. Magnesium

Magnesium is an essential nutrient with key physiological functions in muscle and nerve function, bone health among others. This mineral is widely present in food and hence deficiency is rare. RDI values are set between 80-420 mg/day for the general population excluding infants. As the consumption of magnesium has not been shown to produce toxic effects when ingested through food, there are few reports to support the establishment of ULs for magnesium. However, upper level of magnesium intake recommendations from supplements have been set for populations groups as per Table 36.

Table 36. RDI, EAR and UL (mg/day) for magnesium in population groups stratified by age					
Age	**RDI (mg/day)**		**EAR (mg/day)**		**UL (mg/day)**
	Males	**Females**	**Males**	**Females**	
≤6 months	30 (AI)	30 (AI)	-	-	ND
7–12 months	75 (AI)	75 (AI)	-	-	ND
1–3 years	80	80	65	65	65
4–8 years	130	130	110	110	110
9–13 years	240	240	200	200	350
14–18 years	410	360	340	300	350
19–30 years	400	310	330	255	350
31-50 years	420	320	350	265	350
51-70 years	420	320	350	265	350
>70 years	420	320	350	265	350

Table 36. RDI, EAR and UL (mg/day) for magnesium in population groups stratified by age					
Age	RDI (mg/day)		EAR (mg/day)		UL (mg/day)
	Males	Females	Males	Females	
Notes: data obtained from (NHMRC, 2006c) *Average of data for males and females AI (Adequate Intake); EAR (Estimated Average Requirement); RDI (Recommended Dietary Intake); UL (Upper Level of Intake); ND (Not Determined)					

The daily magnesium intake obtained from data in the Australian Health Survey: Usual Nutrient Intakes, 2011–12 show that the average and 95th percentile for all age groups is 290 mg/day and 421 mg/day respectively.

Table 37. Estimated daily intake of magnesium from foods in different population groups (mg/day)				
Age	Mean		95th percentile	
	Males	Females	Males	Females
Children 2-3y	223	196	300	267
Children 4-8y	241	212	322	287
Children 9-13y	295	252	444	379
Adolescents 14-18y	322	263	476	396
Adults 19-30	377	286	547	423
Adults 31-50	391	306	567	449
Adults 51-70	366	311	535	455
Adults 71 and over	326	274	486	406
Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015b)				

Table 38 below captures the average concentration of magnesium in several food products. The most important sources of magnesium include different fruits and green vegetables, pumpkin seeds, nuts and some animal products. Cell cultured duck contains levels of magnesium that are comparable to conventional meat and other animal-derived products. Based on the maximum inclusion rate (80%) the average and 95th exposure to magnesium for the Australian population are estimated to be 5.95 mg/day and 11.51 mg/day, respectively. Therefore, cell cultured duck is not expected to negatively affect the consumer. Magnesium is a safe component with no known adverse effects at the levels present in food and, consequently, safe at the concentrations present in cell cultured duck.

Table 38. Magnesium concentration in different foods	
Food commodity	Concentration (mg per 100 g)
Cell cultured duck	22.3
Duck, breast, lean, raw	26
Egg, chicken, yolk, hard-boiled	7
Chicken, breast, lean flesh, raw	31
Chicken, breast, lean flesh, skin & fat, baked, no added fat	29
Beef, boneless dice or strips, lean, raw	24

Table 38. Magnesium concentration in different foods	
Food commodity	**Concentration (mg per 100 g)**
Meat alternative products made from a fungus base	42
Meat alternative, legume and/or vegetable base	48
Seed, pumpkin, hulled & dried	530
Nut, Brazil, raw or blanched, unsalted	350
Nut, almond, with skin, raw, unsalted	266
Milk, cow, powder, skim	110
Cheese, cheddar, processed, regular fat	26
Note: data obtained from the Australian Food Composition Database – Release 2.0	

E.1.2.5. Folate (vitamin B9)

Folate is a naturally occurring form of vitamin B9, which is involved in numerous key physiological functions such as development, red cell formation and cell maintenance. The average RDI for folate is between 150 and 400 µg/day for the general population excluding infants (>3 years) as seen in Table 39. Only AIs are available for infants of 12 months and below. Upper levels of intake for dietary folate equivalents (DFE) from fortified foods or supplements are included in the table below. The UL for folate is 1,000 µg/day for the adult population 19+ years.

Table 39. RDI, EAR and UL (µg/day) for folate in population groups stratified by age			
Age	**RDI (µg/day)**	**EAR (µg/day)**	**UL (µg DFE/day)**
≤6 months	65 (AI)	-	ND
7–12 months	80 (AI)	-	ND
1–3 years	150	120	300
4–8 years	200	160	400
9–13 years	300	250	600
14–18 years	400	330	800
19–30 years	400	320	1,000
31-50 years	400	320	1,000
51-70	400	320	1,000
>70 years	400	320	1,000
Notes: data obtained from (NHMRC, 2006a) *Average of data for males and females AI (Adequate Intake); EAR (Estimated Average Requirement); RDI (Recommended Dietary Intake); UL (Upper Level of Intake); ND (Not Determined)			

As presented in Table 40, this data reports an estimated daily mean intake and 95th percentile of 541 and 833 µg/day for the female population and 670 and 1,026 µg/day for the male population. Some of the most common sources of folate include fruits and vegetables like spinach, enriched foods (e.g., breakfast cereals) and some animal products such as liver.

Table 40. Estimated daily intake of dietary folate equivalents from foods in different population groups (µg/day)

Age	Mean		95th percentile	
	Males	Females	Males	Females
Children 2-3y	523	469	759	690
Children 4-8y	638	575	907	825
Children 9-13y	709	589	1105	913
Adolescents 14-18y	752	558	1154	877
Adults 19-30	667	512	1041	810
Adults 31-50	686	517	1071	818
Adults 51-70	675	548	1057	857
Adults 71 and over	714	565	1115	877

Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015a)

As seen in Table 41, cell cultured duck has a higher concentration of folate (92 µg/100 g) compared to some commonly consumed meat commodities (21-54 µg/100 g). Based on the inclusion rates the average and 95th exposure to folate for the Australian population are estimated to be 22.77 µg/day and 49.55 µg/day, respectively. Considering this, cell cultured duck is not expected to negatively affect the consumer. Moreover, folate intake would still be below the UL in most cases.

Table 41. DFE concentration in different foods

Food commodity	Concentration (µg per 100 g)
Cell cultured duck	92
Duck, lean flesh, baked, no added fat	23
Egg, chicken, yolk, hard-boiled	180
Chicken, breast, lean flesh, grilled, no added fat	54
Chicken, liver, raw	1,450
Beef, boneless dice or strips, lean, raw	21
Meat alternative products made from a fungus base	107
Meat alternative, legume and/or vegetable base	76
Cabbage	425
Spinach, water, fresh, raw	225
Milk, cow, powder, regular fat, unfortified	117

Note: data obtained from the Australian Food Composition Database – Release 2.0

E.1.2.6. Zinc

Zinc is an essential nutrient with key physiological functions in maintaining a healthy immune system, protein metabolism, reproduction and others. The RDI is set between 3-14 mg/day for the general population excluding infants. The UL of zinc is estimated to be 4-40 mg/day depending on the population group as shown in Table 42.

Table 42. RDI, EAR and UL (mg/day) for zinc in population groups stratified by age

Age	RDI (mg/day)		EAR (mg/day)		UL (mg/day)
	Males	Females	Males	Females	
≤6 months	2 (AI)	2 (AI)	-	-	4
7–12 months	3	3	2.5	2.5	5
1–3 years	3	3	2.5	2.5	7
4–8 years	4	4	3	3	12
9–13 years	6	6	5	5	25
14–18 years	13	7	11	6	35
19–30 years	14	6.5	12	8	40
31-50 years	14	6.5	12	8	40
51-70 years	14	6.5	12	8	40
>70 years	14	6.5	12	8	40

Notes: data obtained from (NHMRC, 2006c)
*Average of data for males and females
AI (Adequate Intake); EAR (Estimated Average Requirement); RDI (Recommended Dietary Intake); UL (Upper Level of Intake); ND (Not Determined)

The average and 95th percentile of the zinc daily intake obtained from the Australian Health Survey: Usual Nutrient Intakes, 2011–12 is 9.8 mg/day and 13.8 mg/day, respectively for the Australian population above 2 years old (Table 43).

Table 43. Estimated daily intake of zinc from foods in different population groups (mg/day)

Age	Mean		95th percentile	
	Males	Females	Males	Females
Children 2-3y	7.7	6.7	10.6	9.4
Children 4-8y	8.3	7.4	11.5	10.2
Children 9-13y	10.6	9	15	13.0
Adolescents 14-18y	12.8	8.7	17.7	12.6
Adults 19-30	13.1	8.9	18.0	12.9
Adults 31-50	12.9	9.3	17.9	13.4
Adults 51-70	12.1	9.6	16.8	13.8
Adults 71 and over	11.1	9	15.7	13.0

Note: data obtained from Australian Health Survey: Usual Nutrient Intakes, 2011–12 (ABS, 2015a)

The most important sources of zinc include meat, cereals, seafood, among others. The zinc content in cell cultured duck (2.83 mg/100g) is within the range of zinc contained in conventional meat products (0.87-5.7 mg/100g). Based on the maximum inclusion rate, the average and 95th exposure to zinc for the Australian (above 2 years old) from cell cultured duck are estimated to be 0.76 mg/day and 1.52 mg/day, respectively and far below the UL. Considering this, cell cultured duck is not expected to negatively affect the consumer. Additionally, zinc is a safe component with no known adverse effects at the doses present in food.

Table 44. Zinc concentration in different foods

Food commodity	Concentration (mg per 100 g)
Cell cultured duck	2.83
Duck, breast, lean, raw	2
Egg, chicken, yolk, hard-boiled	2.7
Chicken, breast, lean flesh, raw	0.52
Chicken, breast, lean flesh, skin & fat, baked, no added fat	0.87
Beef, boneless dice or strips, lean, raw	5.7
Oyster, raw	20.25
Prawn, flesh, cooked from raw, no added fat	2.19
Meat alternative, mycoprotein/fungus base, cooked	7.94
Meat alternative, legume and/or vegetable base	0.85
Milk, cow, powder, skim	3.3
Note: data obtained from the Australian Food Composition Database – Release 2.0	

E.1.3. Exposure to media residues

Below, dietary exposure calculations are provided for the three media components that do not have a safe history of use in food. These were pluronic acid, ethanolamine and recombinant bovine insulin. Table 45 presents the exposure to each component taking into account the maximum acceptable limit for each residue listed in the specifications and dividing the mean by the average body weight (79.5 kg) obtained from the average of the mean body weight of males and females reported in the National Health Survey 2017-18 (ABS, 2018).

Table 45. Estimated exposure to media residues derived from the consumption of cell cultured duck at 80% inclusion rate

Cell cultured duck				
Maximum acceptable limit (mg/kg)	**Mean Intake (mg/kg BW/day)**	**Margin of exposure (mean)**	**95th percentile (mg/kg BW/day)**	**Margin of exposure (95th percentile)**
Pluronic acid				
500	0.169	88.77	0.338	44.37
Ethanolamine				
30	0.010	295.54	0.020	147.77
Bovine Insulin				
2	0.000677	-	0.001353	-

E.1.3.1. Pluronic acid

This substance is one of three media substances with limited history of safe use in food. Consequently, the exposure from current uses has been estimated to calculate the overall exposure from all dietary and other sources. More information on the uses and toxicological profile of the substance can be found in Section C.2.5.5. and Appendix 21. Non-dietary sources include cosmetics and medicines. Cosmetics have not been considered as the compound is not absorbed through skin due to its high molecular mass. The intake derived from medicines containing pluronic acid could not be estimated based on the lack of available data. This limitation in the assessment is not expected to have a significant impact as the exposure derived from medicines containing pluronic acid is likely to be marginal.

Based on the inclusion rates and the mean and 95th percentile exposure to pluronic acid are estimated to be 0.169 mg/kg BW/day and 0.338 mg/kg BW/day, respectively.

An assessment of this compound and its exposure can be found in Section C.2.2.3.2. Pluronic Acid with further information in Appendix 21. To summarize, results show that the levels of pluronic acid ranged between 177.6 mg/kg and 367.9 mg/kg (average: 292.86 mg/kg). The estimated exposure to pluronic acid derived from cell cultured duck can be found in Table 45. The Margin of Exposure (MOE) (calculated by dividing the ADI 15 mg/kg BW/day) by the estimated intake) is presented in the same table. The MOE is used by risk assessors as a tool to consider possible safety concerns arising from the presence of a substance in food in the absence of an established ADI or a tolerable daily intake (TDI). The MOE indicates if an intake is likely to be harmful or not, with a low MOE representing a greater risk than a high MOE (EFSA, 2023). The MOE population is determined as 88.77 and 44.37 (mean and 95th percentile, respectively).

Based on the analytical data and the risk assessment performed (Section C.2.2.3.2. Pluronic Acid) exposure to pluronic acid derived from cell cultured duck can be considered safe.

E.1.3.2. Ethanolamine

Exposure to ethanolamine (EA) derived from the consumption of cell cultured duck has been estimated following the same methodology for pluronic acid. This substance is one of the key media substances with a limited history of safe use in food. Consequently, the exposure from current uses has been estimated to calculate the overall exposure from all dietary and other sources. More information on the uses and toxicological profile of the substance can be found in Section C.2.2.2.3. Ethanolamine and Appendix 21. Non-dietary sources include food contact materials and dietary sources may include lecithin-rich products among others. The intake derived from these sources had not been considered in the assessment based on the lack of available data. This limitation in the assessment is not expected to have a significant impact as the exposure derived from these sources is likely to be marginal.

Based on the inclusion rates and the mean and 95th percentile exposure to ethanolamine are estimated to be 0.01 mg/kg BW/day and 0.02 mg/kg BW/day, respectively.

An assessment of this compound and its exposure can be found in Section C.2.2.2.3. Ethanolamine. To summarize, the analytical data from five independent batches show that the levels of EA ranged between 3.0 and 13.3 µg/g (average: 6.94 µg/g). The estimated exposure to EA and the MOE can be found in Table 45. The MOE for determined as 295.54 and 147.77 (mean and 95th percentile, respectively).

Based on the analytical data and the risk assessment performed, exposure to EA derived from cell cultured duck can be considered safe.

E.1.3.3. Bovine insulin

Exposure to insulin derived from the consumption of cell cultured duck has been estimated following the same methodology presented for pluronic acid. This substance is one of the key media substances with limited history of safe use in food. Consequently, the exposure from current uses has been estimated to calculate the overall exposure from all dietary and other sources. More information on the uses and toxicological profile of the substance can be found in Section C.2.1.4. Non-dietary sources include medicines

(injectable and oral insulin), and dietary sources include non-processed animal products. Dietary sources are negligible compared to the exposure to insulin from medicinal uses.

Based on the inclusion rates the mean and 95th percentile exposure to insulin is estimated to be 0.000677 mg/kg BW/day and 0.001353 mg/kg BW/day, respectively.

An assessment of this compound and its exposure can be found in Section C.2.1.4. To summarize, recombinant bovine insulin was below the LOQ (2 µg/g) in five batches of cell cultured duck (below the LOD of 0.5 µg/g in batch). No safety or toxicological limits exist for this substance. As such an MOE could not be derived. However, in the unlikely event of bovine insulin being present in the cell cultured duck, it would be digested and absorbed as peptides and amino acids. Consequently, the use of recombinant bovine insulin in the production process does not introduce any food safety risks.

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

The addition of cell cultured duck to food does not confer any potential beneficial physiological or health-related outcome. As described in Section C, the safety of the cells has been thoroughly evaluated. Toxigenicity deriving from the source of the novel food is considered highly unlikely due to the lack of toxigenic potential arising from duck meat consumption and therefore by extension duck cells which have been fully characterised. Analysis of the composition of cell cultured duck has not shown any new concerning constituents outside of safe limits. The culture media used in the production process is chemically defined and composed of food-safe components with findings from extensive literature reviews having identified no toxicological risks on media residues. The exposure assessment shown in Part E demonstrates that its consumption will not be nutritionally disadvantageous.

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

F.1. Demonstrate the level of consumer awareness and understanding of the novel food or novel food ingredient

Different market research studies have been conducted worldwide to establish the degree of awareness and understanding of the average consumer about cultivated meat. However, only a few studies have focused on the awareness among Australian and New Zealand consumers.

Cultured meat is expected to provide consumer with an alternative to conventional meat and plant-based meat analogues. It will provide a more similar texture and taste to their conventional counterpart than the current alternatives available in the market. While this product is not intended to fully replace conventional duck meat, it is expected to partially displace the consumption of conventional meat and meat analogues.

It should be noted that a consolidated nomenclature will be needed to legally name this category and give the consumer with key information about the nature of the food. We propose the use of the term ‘cultured’ as this is understandable to the English-speaker and trustfully and transparently indicates that the product is cell-derived. If the product is labelled as ‘cultured’, consumers will be able to identify its nature and, consequently, make an informed choice. It is on our best interest to clearly state that the product is derived from cells as the ethical and sustainability contributions of these products are expected to play an important role in consumers’ choice making.

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

Bogueva and Marinova (2020) found that 28% of their Australian surveyed respondents between 18 and 24 years old were willing to try cultured meat, whilst the remaining 72% showed uncertainty due to different factors such as taste, health and socio-cultural impact. These results were also in line with those obtained by (Garcez de Oliveira Padilha et al., 2022). The researchers reported that 24-27% of the respondents were willing to try cultivated beef or chicken. However, the products obtained a negative score on the different

attributes (health, taste, safe, affordability and benefits for the environment) except for animal welfare, where most respondents saw a benefit in consuming cultivated meat.

In New Zealand, Giezenaar et al. (2023) et al. reported that 41% of their respondents (all between 25 and 55 years and reported meat consumers) had a positive opinion about cultivated meat. Plant-based meat eaters were more prone to having a positive image about cultivated meat than those that did not consume these products in their diets. Approximately 51% of the respondents showed a neutral attitude towards cultivated meat, and as little as 9% had a negative perception of cultivated meat.

It should be noted that New Zealand and Australia are strongly conservative regions as meat consumption is deeply embedded in their culture and is seen as a fundamental element of their traditional diet. Consequently, new products such as cultivated meat will first need to be placed in the market to increase awareness and responsibly inform the consumers about its characteristics and benefits for the environment.

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)

For additional information on the safety and nutritional adequacy of the ingredient, please refer to Sections D and E. Based on the information provided in these sections, the product is not expected to have any adverse effect on the population regardless of age, group or health status. Consumers are expected to mimic the consumption patterns of the conventional counterparts. "Since the final products will be manufactured to be nutritionally comparable to their conventional counterparts, no nutritional imbalances are expected.

References

EUROPEAN COMMISSION, Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. *OJ L 139 30.4.2004, p. 1*.

EUROPEAN COMMISSION, Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *OJ L 139 30.4.2004, p. 55*.

EUROPEAN COMMISSION, Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. *OJ L 327 11.12.2015, p. 1*.

AALBERSE, R. C. 2000. Structural biology of allergens. *J Allergy Clin Immunol,* 106**,** 228-38.

ABDELMOTELEB, M., ZHANG, C., FUREY, B., KOZUBAL, M., GRIFFITHS, H., CHAMPEAUD, M. & GOODMAN, R. E. 2021. Evaluating potential risks of food allergy of novel food sources based on comparison of proteins predicted from genomes and compared to www.AllergenOnline.org. *Food and Chemical Toxicology,* 147**,** 111888.

ABS 2015a. 43640DO003_20112012 Australian Health Survey: Usual Nutrient Intakes, 2011–12 — Australia.

ABS 2015b. Australian Bureau of Statistics 43640DO04_20112012 Australian Health Survey: Usual Nutrient Intakes, 2011–12 — Australia.

ABS 2015c. Australian Bureau of Statistics 43640DO005_20112012 Australian Health Survey: Nutrition First Results – Foods and Nutrients, 2011–12 — Australia.

ABS 2018. Australian Bureau of Statistics, National Health Survey: First results.

AHA 2015. Animal Health Australia, Disease strategy: Classical swine fever (Version 4.0). Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 4, National Biosecurity Committee, Canberra, ACT.

AHA 2022. Animal Health Australia, Response strategy: African swine fever (version 5.1). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 5, Canberra, ACT.

ALCáNTARA VILLAR, M., PALACIOS COLOM, L., ANAYA ANAYA, S., BUSTAMANTE ORVAY, L. & JIMENO NOGALES, L. 2019. Duck Egg Allergy in an Adult Patient Without Allergy to Chicken Egg. *J Investig Allergol Clin Immunol,* 29**,** 245-246.

ANDRé, F., CAVAGNA, S. & ANDRé, C. 2003. Gelatin prepared from tuna skin: a risk factor for fish allergy or sensitization? *Int Arch Allergy Immunol,* 130**,** 17-24.

ANET, J., BACK, J. F., BAKER, R. S., BARNETT, D., BURLEY, R. W. & HOWDEN, M. E. H. 1985. Allergens in the White and Yolk of Hen's Egg. *International Archives of Allergy and Immunology,* 77**,** 364-371.

AñíBARRO, B., SEOANE, F. J., VILA, C. & LOMBARDERO, M. 2000. Allergy to eggs from duck and goose without sensitization to hen egg proteins. *J Allergy Clin Immunol,* 105**,** 834-6.

ANSES-CIQUAL 2020. ANSES-CIQUAL French food composition table version 2020.

ARBIT, E. & KIDRON, M. 2017. Oral Insulin Delivery in a Physiologic Context: Review. *Journal of Diabetes Science and Technology,* 11**,** 825-832.

ARIAS, M. & FAN, H. 2014. The saga of XMRV: a virus that infects human cells but is not a human virus. *Emerg Microbes Infect,* 3**,** e.

BAI, J. P., CHANG, L. L. & GUO, J. H. 1996. Effects of polyacrylic polymers on the degradation of insulin and peptide drugs by chymotrypsin and trypsin. *J Pharm Pharmacol,* 48**,** 17-21.

BANDYOPADHYAY, A., KOSANAM, H., YANG, R. S., GUPTA, B., NARALAKATTU, N., PAKHALE, S., KRESS, J., RICHARDSON, D. & LY, J. 2022. Low-molecular-weight impurity in Poloxamer 188 responsible for atypical cell culture performance for mAb production. *Journal of Biotechnology,* 351**,** 13-22.

BENDAYAN, M., ZIV, E., BEN-SASSON, R., BAR-ON, H. & KIDRON, M. 1990. Morpho-cytochemical and biochemical evidence for insulin absorption by the rat ileal epithelium. *Diabetologia,* 33**,** 197-204.

BIRUETE, A., HILL GALLANT, K. M., LLOYD, L., MEADE, A., MOE, S. M., ST-JULES, D. E. & KISTLER, B. M. 2023. 'Phos'tering a Clear Message: The Evolution of Dietary Phosphorus Management in Chronic Kidney Disease. *Journal of Renal Nutrition,* 33**,** S13-S20.

BOGUEVA, D. & MARINOVA, D. 2020. Cultured Meat and Australia's Generation Z. *Front Nutr,* 7**,** 148.

BOTTJE, W. G., LASSITER, K., DRIDI, S., HUDSON, N. & KONG, B. W. 2017. Enhanced expression of proteins involved in energy production and transfer in breast muscle of pedigree male broilers exhibiting high feed efficiency. *Poult Sci,* 96**,** 2454-2458.

CAHEN, FRITSCH & WÜTHRICH 1998. Food allergy with monovalent sensitivity to poultry meat. *Clinical & Experimental Allergy,* 28**,** 1026-1030.

CAPUA, I. & ALEXANDER, D. J. 2002. Avian influenza and human health. *Acta Trop,* 83**,** 1-6.

CARINO, G. P. & MATHIOWITZ, E. 1999. Oral insulin delivery1Abbreviations: GI, gastrointestinal; IDDM, insulin-dependent diabetes mellitus; IU, international units; NIDDM, non-insulin-dependent diabetes mellitus; PIN, phase inversion nanoencapsulation; ZOT, zona occludens toxin.1. *Advanced Drug Delivery Reviews,* 35**,** 249-257.

CARO CONTRERAS, F. J., GINER MUñOZ, M. T., MARTIN MATEOS, M. A., PLAZA MARTIN, A. M., SIERRA MARTINEZ, J. I. & LOMBARDERO, M. 2008. Allergy to quail's egg without allergy to chicken's egg. Case report. *Allergologia et Immunopathologia,* 36**,** 234-237.

CAUJOLLE, F. M. E., CAUJOLLE, D. H., CROS, S. B. & CALVET, M. M. J. 1967. LIMITS OF TOXIC AND TERATOGENIC TOLERANCE OF DIMETHYL SULFOXIDE. *Annals of the New York Academy of Sciences,* 141**,** 110-125.

CCC001 2022. Pre-Market Consultation to Support the Safety of Good Meat´s Cultured Chicken as a human Food Ingredient.

CDC 2015. Notes from the Field: Campylobacteriosis Outbreak Associated with Consuming Undercooked Chicken Liver Pâté — Ohio and Oregon, December 2013–January 2014. *Morbidity and Mortality Weekly Report (MMWR).*

CDC. 2022. *Avian Influenza (Bird Flu)* [Online]. Available: https://www.cdc.gov/niosh/topics/avianflu/default.html#:~:text=Infected%20birds%20shed%20bird%20flu,mouth%2C%20eyes%2C%20or%20nose. [Accessed 04/26/2023].

CHANG, L. L. & BAI, J. P. 1996. Evidence for the existence of insulin-degrading enzyme on the brush-border membranes of rat enterocytes. *Pharm Res,* 13**,** 801-803.

CHANG, T.-Y. & HU, M.-L. 1996. Concentrations and lipid peroxidation in tissues and toxicity of para-aminobenzoic acid fed to rats in drinking water. *The Journal of Nutritional Biochemistry,* 7**,** 408-413.

CHONG, M., LEUNG, A. & FERNANDEZ, T. M. 2024. On-site sensory experience boosts acceptance of cultivated chicken. *Future Foods,* 9**,** 100326.

CIR 1983a. 8 Final Report on the Safety Assessment of Triethanolamine, Diethanolamine, and Monoethanolamine. *Journal of the American College of Toxicology,* 2**,** 183-235.

CIR 1983b. Final Report on the Safety Assessment of Triethanolamine, Diethanolamine, and Monoethanolamine. *Journal of the American College of Toxicology,* 2**,** 183-235.

CIR 2008. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. *Int J Toxicol,* 27 Suppl 2**,** 93-128.

COLLINS, M. T. 1997. Mycobacterium paratuberculosis: a potential food-borne pathogen?

COMAI & SULLIVAN 1980. Antiobesity activity of pluronic L-101. *Int J Obes . 1980;4(1):33-42.*

COMMITTEE FT/8, P. F. F. C. 1999. Australian Standard Plastics materials for food contact use.

COUNCIL, N. R. 1984. *Emergency and Continuous Exposure Limits for Selected Airborne Contaminants: Volume 2,* Washington, DC, The National Academies Press.

COUNCIL, N. R. 2007. *Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants: Volume 1,* Washington, DC, The National Academies Press.

CXC1-1969 2022. Codex Alimentarius, General principles of food hygiene CXC 1-1969.

DAFF 2024a. Australian Government Department of Agriculture, Fisheries and Forestry - National list of notifiable animal diseases.

DAFF 2024b. Avian Influenza or Bird Flu - Human health information.

DAVIS, T. L., WALKER, J. R., CAMPAGNA-SLATER, V., FINERTY, P. J., PARAMANATHAN, R., BERNSTEIN, G., MACKENZIE, F., TEMPEL, W., OUYANG, H., LEE, W. H., EISENMESSER, E. Z. & DHE-PAGANON, S. 2010. Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. *PLoS Biol,* 8**,** e1000439.

DEBEER, J., BELL, J. W., NOLTE, F., ARCIERI, J. & CORREA, G. 2021. Histamine Limits by Country: A Survey and Review. *Journal of Food Protection,* 84**,** 1610-1628.

DIMA, R. I. & THIRUMALAI, D. 2002. Exploring the propensities of helices in PrP(C) to form beta sheet using NMR structures and sequence alignments. *Biophys J,* 83**,** 1268-80.

DUCKWORTH, W. C., BENNETT, R. G. & HAMEL, F. G. 1998. Insulin Degradation: Progress and Potential*. *Endocrine Reviews,* 19**,** 608-624.

DUMONT, F., MARECHAL, P. A. & GERVAIS, P. 2006. Involvement of two specific causes of cell mortality in freeze-thaw cycles with freezing to -196 degrees C. *Appl Environ Microbiol,* 72**,** 1330-5.

DURAK-DADOS, A., MICHALSKI, M. & OSEK, J. 2020. Histamine and Other Biogenic Amines in Food. *J Vet Res,* 64**,** 281-288.

EC 2006. Scientific Committee on Consumer Products (SCCP) Opinion on 4-Aminobenzoic acid (PABA).

ECDC 2021. The risk of variant Creutzfeldt-Jakob disease transmission via blood and plasma-derived medicinal products manufactured from donations obtained in the United Kingdom.

ECHA 2023. Dimethyl sulfoxide: Registration Dossier. European Chemicals Agency.

EFSA 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal,* 8**,** 1637.

EFSA 2015. EFSA explains risk assessment: Acrylamide in food.

EFSA 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal,* 16**,** e05206.

EFSA 2020. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. *FSAJournal 2020;18(7):6174, 42 pp*.

EFSA. 2023. *Margin of Exposure* [Online]. Available: https://www.efsa.europa.eu/en/topics/topic/margin-exposure#:~:text=What%20is%20the%20margin%20of,possible%20to%20establish%20a%20HBGV. [Accessed].

EFSA 2024. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal,* 22**,** e8912.

EFSA PANEL ON DIETETIC PRODUCTS, N. & ALLERGIES 2014. Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA Journal,* 12**,** 3894.

EFSA PANEL ON DIETETIC PRODUCTS, N., PRODUCTS, A., NUTRITION, , A., TURCK, D., BRESSON, J.-L., BURLINGAME, B., DEAN, T., FAIRWEATHER-TAIT, S., HEINONEN, M., HIRSCH-ERNST, K. I., MANGELSDORF, I., MCARDLE, H. J., NASKA, A., NEUHäUSER-BERTHOLD, M., NOWICKA, G., PENTIEVA, K., SANZ, Y., SIANI, A., SJöDIN, A., STERN, M., TOMé, D., VINCETI, M., WILLATTS, P., ENGEL, K.-H., MARCHELLI, R., PöTING, A., POULSEN, M., SALMINEN, S., SCHLATTER, J., ARCELLA, D., GELBMANN, W., DE SESMAISONS-LECARRé, A., VERHAGEN, H. & VAN LOVEREN, H. 2021. Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (Revision 1)2. *EFSA Journal,* 19**,** e06555.

EMEA. 2003. Note for Guidance on the use of Bovine Serum in the manufacture of human biological medicinal products.

FALKINHAM, I. J. O. 2009. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *Journal of Applied Microbiology,* 107**,** 356-367.

FAO 2001. Food Balance Sheets: A Handbook, Annex I Food Composition Tables.

FAO 2023. Food Safety Aspects of Cell-based Food.

FARZANEH, M., KHOSHNAM, S. & MOZDZIAK, P. 2017. Concise review: avian multipotent stem cells as a novel tool for investigating cell-based therapies. *J Dairy Vet Anim Res.,* 5**,** 1-4.

FDA 2006. Guidance for Industry: Estimating Dietary Intake of Substances in Food. *In:* (CFSAN), C. F. F. S. A. A. N. (ed.).
FDA 2007. Application number 22-188 (LCP-Fenochol or fenofibrate). Center for Drug Evaluation and Research, FDA.
FDA 2017. Non-Clinical Review, Application number 209139Orig1s000 (Prexxartan, Valsartan). Center for Drug Evaluation and Research, FDA.
FERNáNDEZ CORTéS, S., FERNáNDEZ GARCíA, A., ARMENTIA MEDINA, A. & PINEDA, F. 2013. Duck egg allergy in a patient who tolerates hen's eggs. *J Investig Allergol Clin Immunol,* 23**,** 135-6.
FINDEISEN, P., MüHLHAUSEN, S., DEMPEWOLF, S., HERTZOG, J., ZIETLOW, A., CARLOMAGNO, T. & KOLLMAR, M. 2014. Six subgroups and extensive recent duplications characterize the evolution of the eukaryotic tubulin protein family. *Genome Biol Evol,* 6**,** 2274-88.
FIUME, M. M., HELDRETH, B. A., BERGFELD, W. F., BELSITO, D. V., HILL, R. A., KLAASSEN, C. D., LIEBLER, D. C., MARKS, J. G., SHANK, R. C., SLAGA, T. J., SNYDER, P. W. & ANDERSEN, F. A. 2015. Safety Assessment of Ethanolamine and Ethanolamine Salts as Used in Cosmetics. *International Journal of Toxicology,* 34**,** 84S-98S.
FONTE, P., ARAÚJO, F., REIS, S. & SARMENTO, B. 2013. Oral insulin delivery: how far are we? *J Diabetes Sci Technol,* 7**,** 520-531.
FRITSCHE, P. J., HELBLING, A. & BALLMER-WEBER, B. K. 2010. Vaccine hypersensitivity--update and overview. *Swiss Med Wkly,* 140**,** 238-46.
FSA 2023. Food Standards Agency: Hazard identification: Identification of hazards in meat products manufactured from cultured animal cells.
FSANZ 2021. F2015L00411 Australia New Zealand Food Standards Code – Standard 1.6.1 – Microbiological limits in food.
FSANZ 2022. Compendium of Microbiological Criteria for Food.
FSANZ 2023a. F2023C00821 (C04) Australia New Zealand Food Standards Code - Standard 3.2.1 - Food Safety Programs (Australia Only).
FSANZ 2023b. STANDARD 3.2.2
FOOD SAFETY PRACTICES
AND GENERAL REQUIREMENTS.
FSANZ 2024a. Application Handbook.
FSANZ 2024b. Campylobacter in food.
FSANZ 2024c. F2024C00139 Australia New Zealand Food Standards Code – Schedule 19 – Maximum levels of contaminants and natural toxicants.
FU, T. J., ABBOTT, U. R. & HATZOS, C. 2002. Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid-a comparative study. *J Agric Food Chem,* 50**,** 7154-60.
GARCEZ DE OLIVEIRA PADILHA, L., MALEK, L. & UMBERGER, W. J. 2022. Consumers' attitudes towards lab-grown meat, conventionally raised meat and plant-based protein alternatives. *Food Quality and Preference,* 99**,** 104573.
GARCíA-MENDíVIL, L., MEDIANO, D. R., HERNAIZ, A., SANZ-RUBIO, D., VáZQUEZ, F. J., MARíN, B., LóPEZ-PéREZ, Ó., OTERO, A., BADIOLA, J. J., ZARAGOZA, P., ORDOVáS, L., BOLEA, R. & MARTíN-BURRIEL, I. 2021. Effect of Scrapie Prion Infection in Ovine Bone Marrow-Derived Mesenchymal Stem Cells and Ovine Mesenchymal Stem Cell-Derived Neurons. *Animals* [Online], 11.
GIANGRIECO, I., CIARDIELLO, M. A., TAMBURRINI, M., TUPPO, L., RAFAIANI, C., MARI, A. & ALESSANDRI, C. 2023. Comparative Analysis of the Immune Response and the Clinical Allergic Reaction to Papain-like Cysteine Proteases from Fig, Kiwifruit, Papaya, Pineapple and Mites in an Italian Population. *Foods,* 12**,** 2852.
GIBBS, W. J. & HAGEMANN, T. M. 2004. Purified Poloxamer 188 for Sickle Cell Vaso-Occlusive Crisis. *Annals of Pharmacotherapy,* 38**,** 320-324.
GIEZENAAR, C., GODFREY, A. J. R., OGILVIE, O. J., COETZEE, P., WEERAWARNA N.R.P., M., FOSTER, M. & HORT, J. 2023. Perceptions of Cultivated Meat in Millennial and Generation X Consumers Resident in Aotearoa New Zealand. *Sustainability,* 15**,** 4009.

GONZáLEZ-MANCEBO, E., PASTOR, C., GONZáLEZ-DE-OLANO, D., GANDOLFO-CANO, M., MELENDEZ, A., CUESTA, J., ZAPATERO, A. & BARTOLOMé, B. 2011. Identification of allergens in chicken meat allergy. *Journal of Investigational Allergology & Clinical Immunology,* 21**,** 326-327.

GOODMAN, R. E., EBISAWA, M., FERREIRA, F., SAMPSON, H. A., VAN REE, R., VIETHS, S., BAUMERT, J. L., BOHLE, B., LALITHAMBIKA, S., WISE, J. & TAYLOR, S. L. 2016. AllergenOnline: A peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. *Mol Nutr Food Res,* 60**,** 1183-98.

GRANT, J. A., DUPREE, E. & THUESON, D. O. 1977. Complement-mediated release of histamine from human basophils. III. Possible regulatory role of microtubules and microfilaments. *J Allergy Clin Immunol,* 60**,** 306-11.

GRINDEL, J. M., JAWORSKI T FAU - EMANUELE, R. M., EMANUELE RM FAU - CULBRETH, P. & CULBRETH, P. 2002. Pharmacokinetics of a novel surface-active agent, purified poloxamer 188, in rat, rabbit, dog and man. *Biopharm Drug Dispos . 2002;23(3):87-103.*

GUAN, W., WANG, Y., HOU, L., CHEN, L., LI, X., YUE, W. & MA, Y. 2010. Derivation and characteristics of pluripotent embryonic germ cells in duck. *Poult Sci,* 89**,** 312-7.

GUITTET, M., LE COQ, H. & PICAULT, J. P. 1997. [Risk for the transmission of Newcastle disease by contaminated poultry products]. *Rev Sci Tech,* 16**,** 79-82.

GUO, G., PINELLO, L., HAN, X., LAI, S., SHEN, L., LIN, T. W., ZOU, K., YUAN, G. C. & ORKIN, S. H. 2016. Serum-Based Culture Conditions Provoke Gene Expression Variability in Mouse Embryonic Stem Cells as Revealed by Single-Cell Analysis. *Cell Rep,* 14**,** 956-965.

HADI, J. & BRIGHTWELL, G. 2021. Safety of Alternative Proteins: Technological, Environmental and Regulatory Aspects of Cultured Meat, Plant-Based Meat, Insect Protein and Single-Cell Protein. *Foods,* 10**,** 1226.

HAJEB, P. & SELAMAT, J. 2012. A contemporary review of seafood allergy. *Clin Rev Allergy Immunol,* 42**,** 365-85.

HALDER, S., ISLAM, A., MUHIT, M. A., SHILL, M. C. & HAIDER, S. S. 2021. Self-emulsifying drug delivery system of black seed oil with improved hypotriglyceridemic effect and enhanced hepatoprotective function. *Journal of Functional Foods,* 78**,** 104391.

HAMADA, Y., NAGASHIMA, Y. & SHIOMI, K. 2001. Identification of Collagen as a New Fish Allergen. *Bioscience, Biotechnology, and Biochemistry,* 65**,** 285-291.

HAMILTON, K. A., WEIR, M. H. & HAAS, C. N. 2017. Dose response models and a quantitative microbial risk assessment framework for the Mycobacterium avium complex that account for recent developments in molecular biology, taxonomy, and epidemiology. *Water Research,* 109**,** 310-326.

HANSEN, K. S., BALLMER-WEBER, B. K., LüTTKOPF, D., SKOV, P. S., WüTHRICH, B., BINDSLEV-JENSEN, C., VIETHS, S. & POULSEN, L. K. 2003. Roasted hazelnuts--allergenic activity evaluated by double-blind, placebo-controlled food challenge. *Allergy,* 58**,** 132-8.

HARASAWA, R., MIZUSAWA, H., NOZAWA, K., NAKAGAWA, T., ASADA, K. & KATO, I. 1993. Detection and tentative identification of dominant mycoplasma species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions. *Research in Microbiology,* 144**,** 489-493.

HELLWIG, J. & LIBERACKI, A. B. 1997. Evaluation of the pre-, peri-, and postnatal toxicity of monoethanolamine in rats following repeated oral administration during organogenesis. *Fundam Appl Toxicol,* 40**,** 158-62.

HEMMER, W., KLUG, C. & SWOBODA, I. 2016. Update on the bird-egg syndrome and genuine poultry meat allergy. *Allergo Journal International,* 25**,** 68-75.

HENIKOFF, J. G. & HENIKOFF, S. 1996. Using substitution probabilities to improve position-specific scoring matrices. *Comput Appl Biosci,* 12**,** 135-43.

HENIKOFF, S. & HENIKOFF, J. G. 1992. Amino acid substitution matrices from protein blocks. *Proc Natl Acad Sci U S A,* 89**,** 10915-9.

HEUMüLLER-KLUG, S., MAURER, K., TAPIA-LALIENA, M. Á., STICHT, C., CHRISTMANN, A., MöRZ, H., KHASANOV, R., WINK, E., SCHULTE, S., GREFFRATH, W., TREEDE, R.-D., WESSEL, L. M. & SCHäFER, K.-H. 2023. Impact of cryopreservation on viability, gene

expression and function of enteric nervous system derived neurospheres. *Frontiers in Cell and Developmental Biology,* 11.
HOWARD, J. & HYMAN, A. A. 2003. Dynamics and mechanics of the microtubule plus end. *Nature,* 422**,** 753-8.
ICH 1997. Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products Q5D.
INSTITUTE OF MEDICINE 2001. Iron. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* Washington (DC): National Academies Press (US).
IYER, G., DYAWANAPELLY, S., JAIN, R. & DANDEKAR, P. 2022. An overview of oral insulin delivery strategies (OIDS). *International Journal of Biological Macromolecules,* 208**,** 565-585.
JEONG, M.-J., KIM, Y.-C. & JEONG, B.-H. 2021. The First Report of the Prion Protein Gene (PRNP) Sequence in Pekin Ducks (Anas platyrhynchos domestica): The Potential Prion Disease Susceptibility in Ducks. *Genes* [Online], 12.
JEWELL, R. C., KHOR SP FAU - KISOR, D. F., KISOR DF FAU - LACROIX, K. A., LACROIX KA FAU - WARGIN, W. A. & WARGIN, W. A. 1997. Pharmacokinetics of RheothRx injection in healthy male volunteers. *J Pharm Sci. 1997;86(7):808-812***,** 1997;86(7):808-812.
JIN, Y., HE, X., ANDOH-KUMI, K., FRASER, R. Z., LU, M. & GOODMAN, R. E. 2018. Evaluating Potential Risks of Food Allergy and Toxicity of Soy Leghemoglobin Expressed in Pichia pastoris. *Molecular Nutrition & Food Research,* 62**,** 1700297.
KAJITA, E., WAKIYAMA, M., MIURA, K., MIZUMOTO, K., OKA, T., KOMURO, I., MIYATA, T., YATSUKI, H., HORI, K. & SHIOKAWA, K. 2000. Isolation and characterization of Xenopus laevis aldolase B cDNA and expression patterns of aldolase A, B and C genes in adult tissues, oocytes and embryos of Xenopus laevis. *Biochim Biophys Acta,* 1493**,** 101-18.
KALIC, T., KAMATH, S. D., RUETHERS, T., TAKI, A. C., NUGRAHA, R., LE, T. T. K., HUMENIUK, P., WILLIAMSON, N. A., HIRA, D., ROLLAND, J. M., O'HEHIR, R. E., DAI, D., CAMPBELL, D. E., BREITENEDER, H. & LOPATA, A. L. 2020. Collagen-An Important Fish Allergen for Improved Diagnosis. *J Allergy Clin Immunol Pract,* 8**,** 3084-3092.e10.
KAMINSKI, M. 1962. Study of the cross-reactions of hen and duck ovalbumins. Immunochemical relationship between native proteins and preciptating fragments obtained after proteolysis. *Immunology,* 5**,** 322-332.
KANAUJIA, R., BORA, I., RATHO, R. K., THAKUR, V., MOHI, G. K. & THAKUR, P. 2022. Avian influenza revisited: concerns and constraints. *Virusdisease,* 33**,** 456-465.
KELSO, J. M., COCKRELL, G. E., HELM, R. M. & BURKS, A. W. 1999a. Common allergens in avian meats. *J Allergy Clin Immunol,* 104**,** 202-204.
KELSO, J. M., COCKRELL, G. E., HELM, R. M. & BURKS, A. W. 1999b. Common allergens in avian meats. *J Allergy Clin Immunol,* 104**,** 202-4.
KERLETA, V., ANDRLIK, I., BRAUNMüLLER, S., FRANKE, T., WIRTH, M. & GABOR, F. 2010. Poloxamer 188 supplemented culture medium increases the vitality of Caco-2 cells after subcultivation and freeze/thaw cycles. *ALTEX - Alternatives to animal experimentation,* 27**,** 191-197.
KIM, D. S., LEE, M. W., YOO, K. H., LEE, T.-H., KIM, H. J., JANG, I. K., CHUN, Y. H., KIM, H. J., PARK, S. J., LEE, S. H., SON, M. H., JUNG, H. L., SUNG, K. W. & KOO, H. H. 2014. Gene Expression Profiles of Human Adipose Tissue-Derived Mesenchymal Stem Cells Are Modified by Cell Culture Density. *PLOS ONE,* 9**,** e83363.
KISS, Z. & ANDERSON, W. B. 1989. Phorbol ester stimulates the hydrolysis of phosphatidylethanolamine in leukemic HL-60, NIH 3T3, and baby hamster kidney cells. *J Biol Chem,* 264**,** 1483-7.
KLEBERGER, K.-E. 1967. AN OPHTHALMOLOGICAL EVALUATION OP DMSO. *Annals of the New York Academy of Sciences,* 141**,** 381-385.
KLUG, C., HEMMER, W., ROMáN-CARRASCO, P., FOCKE-TEJKL, M., QUIRCE, S., BOYANO-MARTíNEZ, T., GAUBITZER, E., WANK, H. & SWOBODA, I. 2020. Gal d 7—a major allergen in primary chicken meat allergy. *Journal of Allergy and Clinical Immunology,* 146**,** 169-179.e5.

KNAAK, J. B., LEUNG, H.-W., STOTT, W. T., BUSCH, J. & BILSKY, J. 1997. Toxicology of Mono-, Di-, and Triethanolamine. *In:* WARE, G. W. (ed.) *Reviews of Environmental Contamination and Toxicology.* New York, NY: Springer New York.

KRáTKý, M., KONEčNá, K., JANOUšEK, J., BRABLíKOVá, M., JANďOUREK, O., TREJTNAR, F., STOLAříKOVá, J. & VINšOVá, J. 2019. 4-Aminobenzoic Acid Derivatives: Converting Folate Precursor to Antimicrobial and Cytotoxic Agents. *Biomolecules,* 10.

KRETSCHMER, R., FERGUSON-SMITH, M. A. & DE OLIVEIRA, E. H. C. 2018. Karyotype Evolution in Birds: From Conventional Staining to Chromosome Painting. *Genes (Basel),* 9.

KUEHN, A., CODREANU-MOREL, F., LEHNERS-WEBER, C., DOYEN, V., GOMEZ-ANDRé, S.-A., BIENVENU, F., FISCHER, J., BALLARDINI, N., VAN HAGE, M., PEROTIN, J.-M., SILCRET-GRIEU, S., CHABANE, H., HENTGES, F., OLLERT, M., HILGER, C. & MORISSET, M. 2016. Cross-reactivity to fish and chicken meat – a new clinical syndrome. *Allergy,* 71**,** 1772-1781.

KUEHN, A., HILGER, C. & HENTGES, F. 2009. Anaphylaxis provoked by ingestion of marshmallows containing fish gelatin. *J Allergy Clin Immunol,* 123**,** 708-9.

KUEHN, A., HILGER, C., LEHNERS-WEBER, C., CODREANU-MOREL, F., MORISSET, M., METZ-FAVRE, C., PAULI, G., DE BLAY, F., REVETS, D., MULLER, C. P., VOGEL, L., VIETHS, S. & HENTGES, F. 2013. Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. *Clin Exp Allergy,* 43**,** 811-22.

KUROSAWA, H. 2007. Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. *Journal of Bioscience and Bioengineering,* 103**,** 389-398.

LADICS, G. S., CRESSMAN, R. F., HEROUET-GUICHENEY, C., HERMAN, R. A., PRIVALLE, L., SONG, P., WARD, J. M. & MCCLAIN, S. 2011. Bioinformatics and the allergy assessment of agricultural biotechnology products: industry practices and recommendations. *Regul Toxicol Pharmacol,* 60**,** 46-53.

LAND, M. H., PIEHL, M. D. & BURKS, A. W. 2013. Near fatal anaphylaxis from orally administered gelatin capsule. *The Journal of Allergy and Clinical Immunology: In Practice,* 1**,** 99-100.

LANGELAND, T. 1983. A Clinical and Immunological Study of Allergy to Hen's Egg White: VI. Occurrence of Proteins Cross-Reacting with Allergens in Hen's Egg White as Studied in Egg White from Turkey, Duck, Goose, Seagull, and in Hen Egg Yolk, and Hen and Chicken Sera and Flesh. *Allergy,* 38**,** 399-412.

LEAF, C. W. 1967. Toxicology of some non-ionic surfactants. Soap and Chemical Specialties. 43:48. (cited in CIR Expert Panel, 2008).

LI, J., SETTIVARI, R. S. & LEBARON, M. J. 2019. Genetic instability of in vitro cell lines: Implications for genetic toxicity testing. *Environ Mol Mutagen,* 60**,** 559-562.

LI, J., ZHANG, J., LIU, J., ZHOU, Y., CAI, C., XU, L., DAI, X., FENG, S., GUO, C., RAO, J., WEI, K., JARVIS, E. D., JIANG, Y., ZHOU, Z., ZHANG, G. & ZHOU, Q. 2021. A new duck genome reveals conserved and convergently evolved chromosome architectures of birds and mammals. *GigaScience,* 10**,** giaa142.

LIN, Y. & CHEN, G. 2014. *Embryoid body formation from human pluripotent stem cells in chemically defined E8 media.,* Cambridge, Harvard Stem Cell Institute, 2008.

LIPTON, B. A., DAVIDSON, E. P., GINSBERG, B. H. & YOREK, M. A. 1990. Ethanolamine metabolism in cultured bovine aortic endothelial cells. *Journal of Biological Chemistry,* 265**,** 7195-7201.

LIU, C., ZHONG, Y., APOSTOLOU, A. & FANG, S. 2013. Neural differentiation of human embryonic stem cells as an in vitro tool for the study of the expression patterns of the neuronal cytoskeleton during neurogenesis. *Biochemical and Biophysical Research Communications,* 439**,** 154-159.

LIU, H., FAN, Y., ZHONG, J., MALKOCH, M., CAI, Z. & WANG, Z. 2023. Advance in oral delivery of living material. *Biomedical Technology,* 3**,** 26-39.

LIU, R., YANG, E., LIU, C. & XUE, W. 2012. Tilapia (Oreochromis mossambicus) allergens characterized by ELISA, SDS-PAGE, 2D gels, Western blotting and MALDI-TOF mass spectrometry. *Int J Food Sci Nutr,* 63**,** 259-66.

LOPES, M. H. & SANTOS, T. G. 2012. Prion potency in stem cells biology. *Prion,* 6**,** 142-6.

LöSER, C. 2000. Polyamines in human and animal milk. *Br J Nutr,* 84 Suppl 1**,** S55-8.

LUCHTMAN-JONES, L., FILES, B., BALLAS, S., SWERDLOW, P., BENJAMIN, L., HILLIARD, L., ABBOUD, M., WOJTOWICZ-PRAGA, S. & GRINDEL, J. 2000. *Phase I study of Flocor in patients with acute chest syndrome of sickle cell disease*.

LUO, Y., DE SOUZA, C., RAMACHANDRAN, M., WANG, S., YI, H., MA, Z., ZHANG, L. & LIN, K. 2022. Precise oral delivery systems for probiotics: A review. *Journal of Controlled Release,* 352**,** 371-384.

LUPATOV, A. Y. & YARYGIN, K. N. 2022. Telomeres and Telomerase in the Control of Stem Cells. *Biomedicines,* 10.

MANKES, R. F. 1986. Studies on the embryopathic effects of ethanolamine in Long-Evans rats: preferential embryopathy in pups contiguous with male siblings in utero. *Teratog Carcinog Mutagen,* 6**,** 403-17.

MARSCHüTZ, M. K., CALICETI, P. & BERNKOP-SCHNÜRCH, A. 2000. Design and In Vivo Evaluation of An Oral Delivery System for Insulin. *Pharmaceutical Research,* 17**,** 1468-1474.

MCALLISTER, J., GREGORY, J., ADAMOPOULOS, J., WALSH, M., STYLIANOPOULOS, A., ARNOLD, A. L., STAFFORD, R., ANDERSSON, P. & STEWART, T. 2023. A foodborne outbreak of campylobacteriosis at a wedding - Melbourne, Australia, 2022. *Commun Dis Intell (2018),* 47.

MICHAEL, A. J. 2016. Polyamines in Eukaryotes, Bacteria, and Archaea. *J Biol Chem,* 291**,** 14896-903.

MICOZZI, S., BARTOLOMé, B., SANCHíS-MERINO, M., ALFAYA, T., ALDUNATE, T., DIAZ, M. & PASTOR-VARGAS, C. 2016. Hypersensitivity to Quail Egg Proteins: What About Hen Egg? *Journal of Investigational Allergology and Clinical Immunology,* 26**,** 316-318.

MILLER, P. J. 2018. *Newcastle Disease in Poultry* [Online]. Available: https://www.msdvetmanual.com/poultry/newcastle-disease-and-other-paramyxovirus-infections/newcastle-disease-in-poultry [Accessed 12/02/2023].

MIRANDA, A., PERICUESTA, E., RAMíREZ, M. & GUTIERREZ-ADAN, A. 2011. Prion protein expression regulates embryonic stem cell pluripotency and differentiation. *PLoS One,* 6**,** e18422.

MOGHTADERI, M., NABAVIZADEH, S. H. & HOSSEINI TESHNIZI, S. 2020. The frequency of cross-reactivity with various avian eggs among children with hen's egg allergy using skin prick test results: fewer sensitizations with pigeon and goose egg. *Allergologia et Immunopathologia,* 48**,** 265-269.

MøRETRø, T. & LANGSRUD, S. 2017. Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. *Comprehensive Reviews in Food Science and Food Safety,* 16**,** 1022-1041.

MUKHTAR, I., MUJEEBAT, I., FADAHUNSI, A. & DIVINE MENSAH, S. 2019. Cyclophilins: The Structure and Functions of an Important Peptidyl-prolyl Isomerase. *International Journal of Biochemistry, Biophysics & Molecular Biology,* 4**,** 1-6.

MUNEGUMI, T. 2015. Aldolase as a Chirality Intersection of L-Amino Acids and D-Sugars. *Origins of Life and Evolution of Biospheres,* 45**,** 173-182.

MUñOZ-ESPARZA, N. C., LATORRE-MORATALLA, M. L., COMAS-BASTÉ, O., TORO-FUNES, N., VECIANA-NOGUÉS, M. T. & VIDAL-CAROU, M. C. 2019. Polyamines in Food. *Front Nutr,* 6**,** 108.

MYKLES, D. L., HAIRE, M. F. & SKINNER, D. M. 2000. Immunocytochemical localization of actin and tubulin in the integument of land crab (Gecarcinus lateralis) and lobster (Homarus americanus). *J Exp Zool,* 286**,** 329-42.

NHMRC 2006a. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes - Folate.

NHMRC 2006b. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes - Iron.

NHMRC 2006c. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes - Magnesium.

NHMRC 2006d. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes - Phosphorus.

NHMRC 2006e. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes - Potassium.
NOEL, P. R., BARNETT, K. C., DAVIES, R. E., JOLLY, D. W., LEAHY, J. S., MAWDESLEY-THOMAS, L. E., SHILLAM, K. W., SQUIRES, P. F., STREET, A. E., TUCKER, W. C. & WORDEN, A. N. 1975. The toxicity of dimethyl sulphoxide (DMSO) for the dog, pig, rat and rabbit. *Toxicology,* 3**,** 143-69.
ONG, K. J., JOHNSTON, J., DATAR, I., SEWALT, V., HOLMES, D. & SHATKIN, J. A. 2021. Food safety considerations and research priorities for the cultured meat and seafood industry. *Comprehensive Reviews in Food Science and Food Safety,* 20**,** 5421-5448.
ÖZOGUL, Y. & ÖZOGUL, F. 2020. Chapter 1 Biogenic Amines Formation, Toxicity, Regulations in Food. *Biogenic Amines in Food: Analysis, Occurrence and Toxicity.* The Royal Society of Chemistry.
PAIN, B., KRESS, C. & RIVAL-GERVIER, S. 2018. Pluripotency in avian species. *Int J Dev Biol,* 62**,** 245-255.
PALI-SCHöLL, I., UNTERSMAYR, E., KLEMS, M. & JENSEN-JAROLIM, E. 2018. The Effect of Digestion and Digestibility on Allergenicity of Food. *Nutrients,* 10.
PAMIES, D., LEIST, M., COECKE, S., BOWE, G., ALLEN, D. G., GSTRAUNTHALER, G., BAL-PRICE, A., PISTOLLATO, F., DE VRIES, R. B. M., HOGBERG, H. T., HARTUNG, T. & STACEY, G. 2022a. Guidance document on Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0).
PAMIES, D., LEIST, M., COECKE, S., BOWE, G., ALLEN, D. G., GSTRAUNTHALER, G., BAL-PRICE, A., PISTOLLATO, F., DE VRIES, R. B. M., HOGBERG, H. T., HARTUNG, T. & STACEY, G. 2022b. Guidance document on Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0). *Altex,* 39**,** 30-70.
PATEL, D. & WITT, S. N. 2017. Ethanolamine and Phosphatidylethanolamine: Partners in Health and Disease. *Oxid Med Cell Longev,* 2017**,** 4829180.
PATRICIA, J. J. & DHAMOON, A. S. 2023. Physiology, Digestion. *StatPearls.* Treasure Island (FL): StatPearls Publishing
Copyright © 2023, StatPearls Publishing LLC.
PEARSON, W. R. 2000. Flexible sequence similarity searching with the FASTA3 program package. *Methods Mol Biol,* 132**,** 185-219.
PEARSON, W. R. 2014. BLAST and FASTA similarity searching for multiple sequence alignment. *Methods Mol Biol,* 1079**,** 75-101.
PEARSON, W. R. 2016. Finding Protein and Nucleotide Similarities with FASTA. *Curr Protoc Bioinformatics,* 53**,** 3.9.1-3.9.25.
PERALTA, O. A., HUCKLE, W. R. & EYESTONE, W. H. 2011. Expression and knockdown of cellular prion protein (PrPC) in differentiating mouse embryonic stem cells. *Differentiation,* 81**,** 68-77.
PIVOVAROVA, O., HÖHN, A., GRUNE, T., PFEIFFER, A. F. H. & RUDOVICH, N. 2016. Insulin-degrading enzyme: new therapeutic target for diabetes and Alzheimer's disease? *Annals of Medicine,* 48**,** 614-624.
REYES, T. F., CHEN, Y., FRASER, R. Z., CHAN, T. & LI, X. 2021. Assessment of the potential allergenicity and toxicity of Pichia proteins in a novel leghemoglobin preparation. *Regul Toxicol Pharmacol,* 119**,** 104817.
RUBIN, L. F. & BARNETT, K. C. 1967. OCULAR EFFECTS OF ORAL AND DERMAL APPLICATION OF DIMETHYL SULFOXIDE IN ANIMALS. *Annals of the New York Academy of Sciences,* 141**,** 333-345.
SAKAGUCHI, M., HORI, EBIHARA, IRIE, YANAGIDA & INOUYE 1999. Reactivity of the immunoglobulin E in bovine gelatin-sensitive children to gelatins from various animals. *Immunology,* 96**,** 286-290.
SAKAGUCHI, M., TODA, M., EBIHARA, T., IRIE, S., HORI, H., IMAI, A., YANAGIDA, M., MIYAZAWA, H., OHSUNA, H., IKEZAWA, Z. & INOUYE, S. 2000. IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *Journal of Allergy and Clinical Immunology,* 106**,** 579-584.
SAMJI, T. 2009. Influenza A: understanding the viral life cycle. *Yale J Biol Med,* 82**,** 153-9.

SáNCHEZ-PéREZ, S., COMAS-BASTÉ, O., COSTA-CATALA, J., IDURIAGA-PLATERO, I., VECIANA-NOGUÉS, M. T., VIDAL-CAROU, M. C. & LATORRE-MORATALLA, M. L. 2022. The Rate of Histamine Degradation by Diamine Oxidase Is Compromised by Other Biogenic Amines. *Front Nutr,* 9**,** 897028.

SCHILLING, R. J. & MITRA, A. K. 1992. Pharmacodynamics of Insulin Following Intravenous and Enteral Administrations of Porcine-Zinc Insulin to Rats. *Pharmaceutical Research,* 9**,** 1003-1009.

SCHNEIDER, I., BAUMGARTNER, W., GRöNINGER, O., STARK, W. J., MäRSMANN, S., CALCAGNI, M., CINELLI, P., WOLINT, P. & BUSCHMANN, J. 2020. 3D microtissue-derived human stem cells seeded on electrospun nanocomposites under shear stress: Modulation of gene expression. *J Mech Behav Biomed Mater,* 102**,** 103481.

SENDOR, A. B., WEERASURIYA, D. & SAPRA, A. 2023. Avian Influenza. *StatPearls.* Treasure Island (FL): StatPearls Publishing

Copyright © 2023, StatPearls Publishing LLC.

SEVILLA, I. A., MOLINA, E., TELLO, M., ELGUEZABAL, N., JUSTE, R. A. & GARRIDO, J. M. 2017. Detection of Mycobacteria by Culture and DNA-Based Methods in Animal-Derived Food Products Purchased at Spanish Supermarkets. *Frontiers in Microbiology,* 8.

SHARMA, S., POLIKS, B., CHIAUZZI, C., RAVINDRA, R., BLANDEN, A. R. & BANE, S. 2010. Characterization of the Colchicine Binding Site on Avian Tubulin Isotype βVI. *Biochemistry,* 49**,** 2932-2942.

SHARP, M. F. & LOPATA, A. L. 2014. Fish Allergy: In Review. *Clinical Reviews in Allergy & Immunology,* 46**,** 258-271.

SHIN, S. & MOORE, T. S., JR. 1990. Phosphatidylethanolamine Synthesis by Castor Bean Endosperm 1: Membrane Bilayer Distribution of Phosphatidylethanolamine Synthesized by the Ethanolaminephosphotransferase and Ethanolamine Exchange Reactions. *Plant Physiology,* 93**,** 154-159.

SILVA, I. C., ROMEIRA, A. M., BARTOLOMé, B. & LEIRIA-PINTO, P. 2009. Egg-bird syndrome in childhood: A case report. *Revista Portuguesa de Imunoalergologia,* 17**,** 283-292.

SINGH-JOY, S. D. & MCLAIN, V. C. 2008. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. *Int J Toxicol,* 27 Suppl 2**,** 93-128.

SMěLá, D., PECHOVá, P., KOMPRDA, T., KLEJDUS, B. & KUBáň, V. 2004. Chromatographic determination of biogenic amines in meat products during fermentation and long-term storage. *Chemicke Listy,* 98**,** 432-437.

SMITH, E. R., HADIDIAN, Z. & MASON, M. M. 1967a. the Single–and Repeated–Dose Toxicity of Dimethyl Sulfoxide. *Annals of the New York Academy of Sciences,* 141**,** 96-109.

SMITH, E. R., MASON, M. M. & EPSTEIN, E. 1967b. THE INFLUENCE OF DIMETHYL SULFOXIDE ON THE DOG WITH EMPHASIS ON THE OPHTHALMOLOGIC EXAMINATION*. *Annals of the New York Academy of Sciences,* 141**,** 386-391.

SNYDER, A. B. & WOROBO, R. W. 2018. Fungal Spoilage in Food Processing. *Journal of food protection,* 81 6**,** 1035-1040.

SOICE, E. & JOHNSTON, J. 2021. Immortalizing Cells for Human Consumption. *International Journal of Molecular Sciences,* 22**,** 11660.

TABOR, C. W., FREEMAN, M. V., BAILY, J. & SMITH, P. K. 1951. STUDIES ON THE METABOLISM OF PARA-AMINOBENZOIC ACID. *The Journal of Pharmacology and Experimental Therapeutics,* 102**,** 98-102.

TAYLOR-ROBINSON, D. 1996. Infections Due to Species of Mycoplasma and Ureaplasma: An Update. *Clinical Infectious Diseases,* 23**,** 671-684.

TGA. 2014. International scientific guideline: Guideline on the use of bovine serum in the manufacture of human biological medicinal products. Available from: https://www.tga.gov.au/resources/resource/international-scientific-guidelines/international-scientific-guideline-guideline-use-bovine-serum-manufacture-human-biological-medicinal-products.

TGA 2018. 2.2 Dimethyl sulfoxide (DMSO) Scheduling medicines and poisons.

THERAPEUTIC GOODS, A. 2021. Notice of interim decisions to amend (or not amend) the current Poisons Standard (oral contraceptive substances). Australian Government. Department of Health and Aged Care.

TOMáS, M. C. A., M. C 1990. Study on the influence of freezing rate on lipid oxidation in fish (salmon) and chicken breast muscles. International Journal of Food Science & Technology, 25, 718-721.

TOMM, J. M., VAN DO, T., JENDE, C., SIMON, J. C., TREUDLER, R., VON BERGEN, M. & AVERBECK, M. 2013. Identification of new potential allergens from Nile perch (Lates niloticus) and cod (Gadus morhua). *J Investig Allergol Clin Immunol,* 23**,** 159-67.

TREICH, N. 2021. Cultured Meat: Promises and Challenges. *Environmental and Resource Economics,* 79**,** 33-61.

TURK, E., WILLS, AIRON A., KWON, T., SEDZINSKI, J., WALLINGFORD, JOHN B. & STEARNS, T. 2015. Zeta-Tubulin Is a Member of a Conserved Tubulin Module and Is a Component of the Centriolar Basal Foot in Multiciliated Cells. *Current Biology,* 25**,** 2177-2183.

UPHOFF, C. C. & DREXLER, H. G. 2014. Detection of Mycoplasma Contamination in Cell Cultures. *Current Protocols in Molecular Biology,* 106**,** 28.4.1-28.4.14.

USEPA, OPP & RD 2006. Reassessment of the One Exemption from the Requirement of a Tolerance for Acetophenone (CAS Re. No. 98-86-2). UNITED STATES ENVIRONMENTAL PROTECTION AGENCY.

VAN REE, R., SAPITER BALLERDA, D., BERIN, M. C., BEUF, L., CHANG, A., GADERMAIER, G., GUEVERA, P. A., HOFFMANN-SOMMERGRUBER, K., ISLAMOVIC, E., KOSKI, L., KOUGH, J., LADICS, G. S., MCCLAIN, S., MCKILLOP, K. A., MITCHELL-RYAN, S., NARROD, C. A., PEREIRA MOURIèS, L., PETTIT, S., POULSEN, L. K., SILVANOVICH, A., SONG, P., TEUBER, S. S. & BOWMAN, C. 2021. The COMPARE Database: A Public Resource for Allergen Identification, Adapted for Continuous Improvement. *Frontiers in Allergy,* 2.

VARRONE, L., GLASS, K., STAFFORD, R. J., KIRK, M. D. & SELVEY, L. 2020. A meta-analysis of case-control studies examining sporadic campylobacteriosis in Australia and New Zealand from 1990 to 2016. *Australian and New Zealand Journal of Public Health,* 44**,** 313-319.

VILLEGAS-CAYLLAHUA, E. A., DUTRA, D. R., DE OLIVEIRA, R. F., PEREIRA, M. R., CAVALCANTI É, N. F., FERRARI, F. B., DE SOUZA, R. A., DE ALMEIDA FIDELIS, H., GIAMPIETRO-GANECO, A., DE SOUZA, P. A., DE MELLO, J. L. M. & BORBA, H. 2024. Concentration of lipids, cholesterol, and fatty acid profile in chicken breast meat affected by wooden breast myopathy frozen for up to 12 mo. *Poult Sci,* 103**,** 103153.

WAITES, K. B., XIAO, L., PARALANOV, V., VISCARDI, R. M. & GLASS, J. I. 2012. Molecular Methods for the Detection of Mycoplasma and Ureaplasma Infections in Humans: A Paper from the 2011 William Beaumont Hospital Symposium on Molecular Pathology. *The Journal of Molecular Diagnostics,* 14**,** 437-450.

WANG, H., LIN, J., LIU, X., LIANG, Z., YANG, P., RAN, P. & LIU, Z. 2016. Identification of α-tubulin, Der f 33, as a novel allergen from Dermatophagoides farinae. *Immunobiology,* 221**,** 911-917.

WANG, Q., ZHANG, L., HU, X., FAN, B., LI, N., LI, H. & WU, C. 2004. Cloning, sequencing and phylogenic analysis of duck prion gene. *Progress in Natural Science,* 14**,** 837-840.

WEREńSKA, M. & OKRUSZEK, A. 2022. Impact of frozen storage on fatty acid profile in goose meat. *Poultry Science,* 101**,** 102213.

WHO 2006. WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies.

WHO. 2018. *Influenza (Avian and other zoonotic)* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/influenza-(avian-and-other-zoonotic) [Accessed 04/26/2023].

WHO/FAO 2009. Codex Alimentarius: Foods derived from modern biotechnology. Second Edition.

WISNIEWSKI, B. T., SHARMA, J., LEGAN, E. R., PAULSON, E., MERRILL, S. J. & MANOGARAN, A. L. 2018. Toxicity and infectivity: insights from de novo prion formation. *Curr Genet,* 64**,** 117-123.

WOAE 2021. World Organization for Animal Health Chapter 11.4. Bovine Spongiform Encephalopathy, Article 11.4.1.

WU, G., MAJEWSKI, J., EGE, C., KJAER, K., WEYGAND, M. J. & LEE, K. Y. C. 2005. Interaction between Lipid Monolayers and Poloxamer 188: An X-Ray Reflectivity and Diffraction Study. *Biophysical Journal,* 89**,** 3159-3173.

WU, Y., GE, C., ZENG, W. & ZHANG, C. 2010. Induced multilineage differentiation of chicken embryonic germ cells via embryoid body formation. *Stem Cells Dev,* 19**,** 195-202.

YAMAMOTO, A., HAYAKAWA, E. & LEE, V. H. L. 1990. Insulin and proinsulin proteolysis in mucosal homogenates of the albino rabbit: Implications in peptide delivery from nonoral routes. *Life Sciences,* 47**,** 2465-2474.

YANG, N., RAY, S. D. & KRAFTS, K. 2014. Cell Proliferation. *In:* WEXLER, P. (ed.) *Encyclopedia of Toxicology (Third Edition).* Oxford: Academic Press.

YEUNG, K. A., CHAI, P. R., RUSSELL, B. L. & ERICKSON, T. B. 2022. Avian Toxins and Poisoning Mechanisms. *J Med Toxicol,* 18**,** 321-333.

ZAI, W. & YUAN, Z. 2024. Chapter 107 - Pathogenesis of viral infection. *In:* TANG, Y.-W., HINDIYEH, M. Y., LIU, D., SAILS, A., SPEARMAN, P. & ZHANG, J.-R. (eds.) *Molecular Medical Microbiology (Third Edition).* Academic Press.

ZHANG, X., DENG, Y., HU, S., HU, X., MA, J., HU, J., HU, B., HE, H., LI, L., LIU, H. & WANG, J. 2023. Comparative analysis of amino acid content and protein synthesis-related genes expression levels in breast muscle among different duck breeds/strains. *Poult Sci,* 102**,** 102277.

ZHOU, W., CHOI, M., MARGINEANTU, D., MARGARETHA, L., HESSON, J., CAVANAUGH, C., BLAU, C. A., HORWITZ, M. S., HOCKENBERY, D., WARE, C. & RUOHOLA-BAKER, H. 2012. HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC-to-EpiSC/hESC transition. *Embo j,* 31**,** 2103-16.