TECHNICAL GUIDANCE NOTES ON THE SAFETY ASSESSMENT OF CULTURED MEAT

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INTRODUCTION

i. Cultured meat refers to meat produced from animal cell culture, and its derived products. It is generally produced by growing selected cell lines or stem cells in a suitable growth medium in a bioreactor. The cells may then be grown on a scaffold as a support to produce products resembling meat muscle.

ii. According to section 54 of the Public Health and Municipal Services Ordinance (Cap. 132), all food available for sale in Hong Kong must be fit for human consumption. Before importing cultured meat into Hong Kong for sale, or manufacturing it locally for human consumption, the trade should first submit data on safety assessment of the cultured meat to the Centre for Food Safety for evaluation. The purpose is to achieve a reasonable certainty that no harm will result from the intended use of the cultured meat and its derived products through scientific safety assessment before their sale in the local market.

iii. This document aims to provide technical guidance to the trade on the suggested requirements regarding the safety assessment of cultured meat. The information listed in the guidance is generally required to support the safety evaluation for a cultured meat or its derived product.

DISCLAIMER

iv. The Technical Guidance Notes are intended for use only as a general reference. The data requirements for the safety assessment of a cultured meat or its derived product should be considered in a case-by-case manner and additional data may be required as appropriate and necessary.

SAFETY ASSESSMENT CRITERIA

Characterisation of the Cultured Meat

1.1 The following information on the identity and source of the cell lines for the production of cultured meat should be included in the safety assessment for the characterisation of the product.

- Identity of the source organism
  - Scientific (Latin) name (family, genus, species, subspecies, breed, if applicable)
  - Accepted synonyms
  - Trivial or common names used to identify the cultured meat intended to be marketed
  - Verification of the identity (e.g. certification, DNA-based authentication)
  - Suitability of the animal sources for human consumption, e.g. any risks of transmissible spongiform encephalopathies
- When using established cell lines: genetic and phenotypic identity and stability of cells
- When using primary cells: biopsy location or source material, cell type(s) isolated, genetic and phenotypic identity of cells
- Information to attest the absence of any risks of infectivity from viruses or other zoonotic agents, e.g. testing for viruses (species-specific viruses), testing for prions in the case of limited health information on source animals
- Information on whether the cells or tissues sourced from a non-GM animal have been genetically modified after biopsy
- Any process(es) applied that may alter the genetic material of the cell lines

Production Process

2.1 A flow chart of the production process, including any quality and safety control checks, should be prepared for the safety assessment of the cultured meat to facilitate the assessment process.

2.2 The key steps and parameters of the production process, including the information on the followings, should be identified.

- Potential by-products;
- Major components and impurities, and comparison of residual anti-microbials, growth promoters and/or modulating factors against levels in published literature;
- Any substance(s) added that may affect human health after consumption;
- Contaminants, including the formation of processing contaminants and a description of the parameters which may lead to the formation of a given processing contaminant;
- Operational limits and key parameters of the production process;
- Measures for production control and quality and safety assurance (e.g. HACCP, GMP, ISO), including critical control points, operational prerequisite programmes, monitored parameters, corrective actions, verification procedures, frequency of analysis, analytical methods, etc.;
- Standardisation criteria (e.g. chemical markers), and;
- Aseptic processing steps established to ensure that the cultured media and cell lines are free from infectious agents (e.g. viruses, bacteria, fungi, prions) throughout cell line selection, cell adaptation, cell proliferation, scaffolding, extraction, concentration and washing.

2.3 The process(es) used in the production of cultured meat that is relatively new to the food producing industry should be characterised and assessed.

2.4 Information related to the cell lines used as listed below should be assessed.

- Background information, identity, and source of cell lines
- Type of cells used as source (e.g. primary cells or established cell lines)
  - If primary cells are used, information on the source, purification steps, cell isolation, cell selection, cell subculture, absence of pathogens and microbial contaminants is to be provided
  - If cells from established cell lines are used, information on the source, the cell line preparation, the cell banking process, as well as the passage number of aliquot of cells used is to be provided
- Any changes made to the cells used (e.g. selection, differentiation, immortalisation, adaptations, reprogramming), and the link of such changes with the production of substances of possible concern
- All processes applied for the treatment, extraction, screening and selection of cell lines or tissues including all chemicals and biological materials used, and the impurities which may result from their use.
- Information on biological agents (e.g. parasites, bacteria, endophytes, viruses, prions) which can infect organisms or tissue cultures used to produce the cultured meat or be hosted by these organisms, and the measures in place to mitigate the respective risks and the impact of these agents on human health
- Information (e.g. biological tests) to show that the cell lines are free from infectious agents (e.g. viruses, bacteria, fungi, prions) where relevant
- Any process(es) applied that may alter the genetic material of the cell line
- The genetic stability of the cells throughout the production process should be investigated by comparison of the starting material (i.e.

initially selected cells from biopsy/cell line) and the cells at different steps of the production process (e.g. propagation step)

- Changes of the morphology, markers of differentiation and other phenotypic features of the cells at the start and at the end of the production process
- Compliance with good cell culture practice and applicable relevant standards, e.g. Guidance Document on Good In vitro Method Practices of the Organisation for Economic Co-operation and Development (OECD), European Medicines Agency's Guidance document on the derivation and characterisation of cell substrates use for production of biotechnological/biological products
- Safety of growth factors of microbial origin (e.g. recombinant proteins, vitamins, amino acids) used in the production

2.5 Information on culture conditions and culture media used as listed below should be assessed.

- Composition of media, including identities and purity of all added substances (e.g. anti-microbials, growth promoters and modulating factors), as well as unintended metabolites that could be potentially produced
- Risk assessments or tests to determine the residue levels for all non-food grade components and potential unintended metabolites present in the culture media
- Safety assessments of biological substances used as media components during production
- Information demonstrating the removal of culture media and/or any added substances used for producing the cultured meat are removed completely
- A risk assessment based on the available toxicity data of the non-food grade components and unintended metabolites, as well as dietary exposure levels arising from the cultured meat, or a comparison of the levels present to that of the same compound

found naturally in conventionally grown meat if these remain in the finished cultured meat

- Information on whether the residue of anti-microbials used would contribute to anti-microbial resistance (AMR) at the levels of exposure anticipated

2.6 Background information, characterisation, and information on the functional role, specifications, quality, purity and safety of all inputs used for production, as well as any potential metabolites whether intended or unintended, including but not limited to the following should be assessed.

- Cell lines or stem cells, and chemicals used for their induction
- Culture media, growth promoters, modulating factors and anti-microbials
- Scaffolding materials, solvents, food additives, enzymes, and processing aids

2.7 Assessment to reasonably demonstrate that genome instability and genetic drift would not lead to the production of undesirable substances in the end-product at levels that can pose a food safety hazard, by strategy (a) combined with strategy (b), or strategy (c) by itself, should be conducted.

(a) By conducting a systematic scientific literature review to identify all known undesirable substances of food safety concern associated with the animal species of the cell culture and establish a list of such substances for subsequent targeted analysis

(b) By performing an in-silico genome screen against relevant databases[1] to establish a list of potential toxins/allergens for subsequent targeted analysis

(c) By carrying out quantitative comparison of the end-product cells

[1] Allergen online databases hosted under the Food Allergy Research and Resources Program
National Centre for Biotechnology Information database
WHO/IUIS Allergen Nomenclature database
The Allergome database
The protein family (Pfam) database
The AllFam database
The Structural Database of Allergenic Proteins (SDAP)
The SWISS-PROT database

against the starter cells through methodologies such as transcriptomics, proteomics or metabolomics so that a list of differentially expressed undesirable substances of food safety concern can be established for subsequent targeted analysis

2.8 Information on any post harvesting handling of the unprocessed cultured meat and further intended processing of the cultured meat, such as transport, drying techniques, storage conditions (duration, light, moisture, temperature) should also be characterised and assessed.

2.9 Any food processing substances used during production/ manufacture which are not intended to be an ingredient of the final product should be identified and assessed.

- If any of these substances is a potential human health hazard, it must be shown that under the proposed intended uses and conditions of consumption, its presence in the final product is at levels that will not cause significant food safety concern.

2.10 In case the dossier contains analytical data on cultured meat batches manufactured by different producers (e.g. the application is submitted by a consortium of producers) or by processes involving steps which can be different, such differences shall be described, equivalency substantiated and consistency in production methods among different producers/processes demonstrated. Food safety management systems should be provided from all producers/processes covering the entire production process. The variability of supplying starting materials is to be investigated and be covered by the analytical data provided. Any changes to the production process during the risk assessment must be notified.

Compositional Data

General requirements

3.1 The compositional data of the cultured meat should include both qualitative and quantitative data. The compositional data should also cover the information on the identities and quantities of impurities or by-products, residues, and chemical and microbiological contaminants such as heavy metals, mycotoxins, polychlorinated biphenyls (PCBs)/dioxins, etc. The type and spectrum of potential target analytes should be considered based on the sources and production process of the cultured meat.

Analytical methods

3.2 For each analytical method employed, full description, references, limit of detection (LOD), and limit of quantification (LOQ) should be documented. Validated methods, preferably nationally or internationally recognised methods (e.g. Association of Official Analytical Chemists, European Pharmacopoeia, International Organization for Standardization, European Committee for Standardization), should be used for the analyses. Certificates of analyses and information on the matrix accreditation and the scope of accreditation of the laboratories should be provided.

3.3 If in-house methods are employed, the analytical protocols implemented should be fully described, and the results of the respective method validation procedures should be provided. If an analytical method is used for a food matrix beyond the scope of accreditation/standardisation, it should be treated as an in-house method (the same applies in cases that standard methods are modified).

3.4 There should be sufficient justification if the analyses are not performed in accredited laboratories.

3.5 A table with all the analytical methods employed and the corresponding analytes should be provided. The table should include the name of the method, the reference, the main analytical technique(s) employed, as well as the respective LOD and/or LOQ.

Addressing compositional variability

3.6 The analytical information should be provided on at least 5 representative batches of the cultured meat which have been independently produced (preferably with independent batches of raw materials), unless a different number of batches is explicitly requested).

3.7 The analyses should preferably be performed on the same group of batches, to obtain a comprehensive picture of their composition. It is expected that the analysed batches are produced either at an industrial production scale or at one representative of it. Representativeness shall be justified.

3.8 The examined batches should be sampled in a manner adequate to address potential compositional variations (e.g. seasonal) of the raw materials. Additional batches of the cultured meat may also be needed to explore the variability of potentially hazardous substances present in the batches of the cultured meat or its source.

3.9 When several production processes are proposed, such data should be provided for each process. Compositional data should also cover the whole variability spectrum of the production process parameters (e.g. range of temperatures applied). The compositional variability should be discussed, highlighting the reasons for the variation in results.

3.10 If the request for safety assessment pertains to various forms of the cultured meat (e.g. dried, frozen), all analyses must be conducted on at least five representative batches of each form, produced independently. Any deviations from this requirement must be justified.

3.11 Analytical data from publications can also be used if the publications provide sufficient information on the laboratory where analyses have been carried out, the methods utilised and if the studies were performed with representative samples of the cultured meat. Available published data can also contribute to providing information on the

variability of the composition of the cultured meat.

Sampling practices

3.12 Principles of representative sampling should be applied (e.g. sample size, containers, conditions), and the rationale on why the employed sampling plan is considered representative should be provided. Information on any relevant standard sampling protocols should be considered and provided. On each certificate of analysis, the name as well as the dates of production and analysis of the batch must be stated.

Compositional analytes

3.13 Information on the identity and quantity of impurities or by-products, residues, and chemical and microbiological contaminants should be provided (e.g. heavy metals, mycotoxins, PCBs/dioxins, pesticides, microbial hygiene indicators and pathogens). The potential target analytes should be selected considering the sources and the production process, regulatory levels as well as the information available in the scientific literature.

3.14 The protein content of the cultured meat should be quantified using the 6.25 nitrogen-to-protein conversion factor. In case the protein content of the cultured meat is substantial (i.e. contributes to at least 12% of the energy content of the cultured meat), it should also be calculated as the sum of the anhydrous amino acids, to account for the presence of non-protein nitrogen and the complete quantitative amino acid profile should be provided. When the cultured meat consists of or is enriched in specific proteins or peptides, characterisation of the individual protein/peptide profile (e.g. sequence, degree of hydrolysis) is additionally requested. Moreover, considering the allergenicity-related analytical requirements, further analyses for the characterisaton of the protein profile may be necessary.

Characterisation of the main constituents and of the naturally or

chemically derived components that characterise the nature of the cultured meat

3.15 A qualitative and quantitative characterisation of the main constituents is to be performed, at least via sum parameters. This should include proximate analyses (i.e. ash, moisture, protein, fat, carbohydrates) and calculation of mass balance. The amount of unidentified components should be indicated and should be as low as possible.

3.16 For the classes of naturally or chemically derived components which characterise the cultured meat (e.g. amino acids, peptides, phospholipids, etc.), comprehensive qualitative and quantitative data should be provided. When it is anticipated that the production process may result in the emergence of new proteins, a thorough characterisation of the protein profile is required.

3.17 Qualitative and quantitative data on nutritionally relevant inherent constituents such as micronutrients, antinutrients and dietary fibre should be provided.

3.18 Information on the occurrence and occurrence levels of inherent substances of possible concern to human health (e.g. possessing toxic, addictive, psychotropic, allergenic potential) should be provided. The impact of processing on the compositional profile of the cultured meat (e.g. occurrence of heat-induced processing contaminants) should also be considered.

3.19 Any substances of concern (e.g. toxins, heavy metals) potentially present in the starting materials, should be analysed in the cultured meat. Particular attention should be given to the possible presence of genotoxic and/or carcinogenic substances in the cultured meat.

3.20 The respective concentration of viable cells and non-viable cells in the cultured meat should be reported.

3.21 In addition to the batch-to-batch analysis, a comprehensive literature search should be performed to retrieve published compositional data (chemical, physiochemical and microbiological) for the source and the part(s) used in/as the cultured meat, as well as for compositional aspects linked to the production process. Information on the keywords and applied inclusion/exclusion criteria for the literature search should be provided. Considering the retrieved information, a rationale on the compositional analysis strategy followed should be provided.

3.22 When it could be relevant to further substantiate the safety, it is recommended that a comparative compositional analysis of the cultured meat to its potential conventional comparators is conducted. While a comparative approach can be useful in some cases, it may not always be adequate for addressing specific risks associated with the cultured meat itself.

Stability testing

Stability of the cultured meat

3.23 During the storage and transport of the cultured meat produced, food safety hazards might arise depending on the stability of the product, thus there is a need to evaluate its stability.

3.24 Stability tests should consider compositional qualifiers, as well as constituents and parameters of the cultured meat which may be susceptible to changes during storage and which may affect its safety and/or its identity or serve as indicators for alterations which could have an impact on the safety and/or the integrity of the cultured meat. The rationale for the parameters selected to be monitored during the stability testing, as well as for those parameters disregarded as not relevant, should be provided.

3.25 Depending on the nature, production process and composition of the cultured meat, the testing is to address the chemical, physiochemical, and microbiological stability of the cultured meat under the intended

conditions of storage, taking into account the effect of packaging and the storage environmental parameters (temperature, light exposure, oxygen, moisture, relative humidity). Information on the intended storage conditions, including the proposed shelf-life, of the cultured meat must be provided as well as on the conditions under which the stability testing was performed.

3.26 The stability testing has to be provided on at least 5 representative batches of the cultured meat that have been independently produced (preferably with independent batches of raw materials). When the request for safety assessment pertains to various forms of the cultured meat (e.g. dried, frozen), such data should be provided for each form. Any deviations from this requirement must be justified. Testing of a lower number of batches should be justified by scientific arguments. The cultured meat batches selected to be monitored at the beginning of the stability testing have to be those monitored for the whole duration of the stability testing. The stability testing results can be taken into consideration when establishing the limits of relevant specification parameters. On the other hand, compliance of the cultured meat with the specification parameters throughout the proposed shelf-life should be demonstrated.

3.27 The monitoring period of the stability test has to cover at least the end of the proposed shelf-life. Intermediate intervals of testing must be considered, depending on the nature of the cultured meat, its composition, as well as the intended shelf-life. Although it is advisable to submit stability testing studies under intended conditions of storage, accelerated conditions may be used as an alternative. Such approaches, usually conducted at higher temperatures, could be applicable only in cases where chemical parameters are monitored. In cases where results from accelerated are extrapolated to predict results under the intended storage conditions, scientific evidence must be provided to justify the validity of this extrapolation. Information on ingredients added to the cultured meat

to improve its stability has to be provided.

Impact of processing on the cultured meat in the proposed-for-use matrices

3.28 If the cultured meat is used as an ingredient added to other foods the manufacture of which requires further processing (e.g. heating), the impact on the cultured meat of this processing is to be investigated. Also alterations in the processed foods due to the presence of the cultured meat should be investigated in foods or in relevant model systems (mimicking the food matrix and the respective processing conditions), taking into consideration at least the extremes of the possible processing conditions (e.g. highest temperature to which the cultured meat will be exposed when used as a food ingredient, lowest and highest pH) as resulting from the proposed uses.

3.29 More specifically, it should be investigated what happens to relevant components of the cultured meat, when it is used as a food ingredient. Interactions with other constituents in the processed foods and the formation of processing contaminants should be investigated. The use of proper controls (e.g. the product manufactured with the same process/recipe without containing the cultured meat as ingredient) is necessary.

3.30 Moreover, when the cultured meat is subject to further processing which differs from the conventionally applied processing methods, any hazards potentially arising are to be identified and characterised.

Specifications

4.1 The chemical, physicochemical, nutritional and microbiological parameters, which characterise and substantiate the identity and safety of the cultured meat, including the respective numerical ranges or limits, should be proposed. A comprehensive set of compositional specification parameters must be provided in a tabulated format.

4.2 Specifications should cover the following information.

- Proximate analytes (protein, lipids, carbohydrates, ash and moisture)
- The major groups of constituents within the cultured meat
- More characteristic components (e.g. micronutrients, number of viable/non-viable microorganisms)
- Parameters relevant for the safety of the cultured meat at the proposed uses and use levels (e.g. toxins, antinutrients, heavy metals, pathogens, impurities or degradation products from the production process)
- Parameters related to the quality and/or stability which may have an impact on the safety of the cultured meat (e.g. markers of lipid oxidation, microbial hygiene indicators or water activity)

4.3 The rationale for each proposed specification parameter and respective limits has to be provided.

4.4 The table must include minimum and/or maximum specification limits for each selected parameter. The specifications, including their limits, should be supported by the available information on the chemical, physiochemical and microbiological composition of the cultured meat including the results from the available batch-to-batch analysis and the stability test. They should be verifiable by means of the analytical techniques as indicated in paragraph 3.2-3.5. Information on the employed analytical techniques and their sensitivity (LOD/LOQ) should be provided.

4.5 In general, the proposed maximum specification limits for undesirable substances should be as low as possible. Existing health-based guidance values (HBGVs) for substances of potential toxicological concern, but also dietary reference values (DRVs) including tolerable upper intake levels (ULs) for micronutrients and exposure estimates to

such compounds, should be considered when proposing the maximum specification limits.

4.6 Minimum specification limits for nutrients may be necessary to ensure that a minimum level is present in the cultured meat, especially when the cultured meat represents a potential alternative or is intended to replace an existing food on the market, which provides a relevant contribution to the intake of certain nutrients.

Proposed Uses and Levels of Consumption

5.1 Information on the proposed uses and anticipated consumption is necessary for the evaluation of food safety, dietary and nutritional significance of the cultured meat.

Target population

5.2 The intended target population of the cultured meat should be specified, whether it is the general population or certain defined population subgroups, such as adolescents, adults. Where it cannot be excluded that the cultured meat intended for a particular group of the population would also be consumed by other groups of the population (e.g. the cultured meat added as an ingredient to foods or be consumed as a whole food), the safety data provided shall also cover those groups.

5.3 In certain cases, the consumption of the cultured meat can be restricted to a particular group of the general population. In such cases, the intended target population (e.g. adults; individuals above 10 years of age; or other age population groups) must be specified.

Proposed uses and use levels

5.4 Information of the proposed uses and use levels should include the following.

- The intended uses of the cultured meat (e.g. as a whole food,

ingredient)

If the cultured meat is intended to be added as an ingredient to foods, the following information should be provided in a tabulated format:

- The food categories in which the cultured meat is proposed to be added
- The proposed maximum use levels of the cultured meat in each food category as consumed (e.g. expressed as mg/kg or mg/100 g or mg/100 mL)
- If the cultured meat is proposed in different forms (e.g. dried, frozen), the food categories and maximum use levels should be proposed for each form of the cultured meat as requested in points above. It should be specified whether the different forms of the cultured meat are meant to be utilised singularly and/or in combination in a specific food category.

5.5 When the cultured meat is intended to be used as a whole food, food(s) already locally consumed which can reasonably reflect the anticipated consumption pattern of the cultured meat should be indicated. The considerations and explanations as to why it is reasonable to expect that the cultured meat corresponds to specific food(s) locally consumed should be provided.

Anticipated intake of the cultured meat

5.6 On the basis of the information provided in paragraph 5.2 to 5.5, the chronic daily intake of the cultured meat should be estimated. This estimate should present both the amount of cultured meat consumed per kilogram body weight and the total absolute amount of cultured meat consumed per day. Mean and high (95^{th} percentile) anticipated daily intakes of the cultured meat for each target population group, including specific population groups such as infants, children, pregnant and lactating women if available, should be provided.

5.7 When estimating the intake, all food categories to which the cultured meat is intended to be added should be considered for a conservative scenario, using individual consumption data from a representative database (e.g. the Second Hong Kong Population-based Food Consumption Survey, EFSA Comprehensive European Food Consumption Database or other national dietary surveys).

5.8 If available toxicological data, human data, data on chemical composition or literature review raise concerns regarding an acute effect, acute intake estimates of the cultured meat should also be considered.

5.9 When the intended uses are expressed as maximum daily intakes of the cultured meat, the maximum daily intake expressed on a per kilogram body weight basis should be provided for each population group of the target population.

5.10 Description of methodological aspects of the intake assessment, which includes the sources of data used (sources of food consumption data and food composition data); scientific principles and methods applied; the assumptions made and their rationale, in particular with respect to the assignment of a food to a particular food category, or with respect to the model used for the calculation of high intake levels should be included. Discussion of the uncertainties related to the assessment, in particular sources of under- or over-estimations should also be covered.

5.11 When the cultured meat is reasonably expected to be used as an alternative to another food already locally consumed (e.g. when the cultured meat is a whole food), the consumption data of food(s) already locally consumed should be used to estimate the anticipated intake of the cultured meat.

5.12 Based on the intended uses of the cultured meat (e.g. whole food, ingredient), the combined intake scenarios resulting from different uses of

the cultured meat should be provided, considering the highest 95th percentile of anticipated intakes of the cultured meat for each group of the target population.

Combined intake considering other sources or its main constituents

5.13 A combined intake of the cultured meat or its main constituent(s) from other sources should be considered. Other potential sources of intake which may derive from other uses (e.g. as a food additive) or from natural occurrence in foods (i.e. from background diet) should be taken into account. Furthermore, when relevant, the combined exposure of constituents from the cultured meat with other potential sources of that constituent (e.g. from the background diet) should be considered. The combined intake should be estimated, taking into account:

- High daily intakes (95th percentile) of the cultured meat/its constituent from the proposed uses and maximum use levels (as estimated in paragraph 5.6-5.12)
- Mean and high daily intakes from natural sources (i.e. from the background diet) derived from literature
- Daily intake from other uses (e.g. food additive) derived from literature

5.14 There might be also cases where the cultured meat is added to foods which may partly replace foods which significantly contribute to the intake of specific compounds (e.g. vitamins, minerals) in the diet. In these cases, the potential double accounting of these compounds from the cultured meat and the background diet should be considered.

5.15 When relevant, considerations on the exposure from fortified foods and/or food supplements and/or other foods already on the market should be provided.

5.16 With regard to nutrients and antinutrients potentially present in the cultured meat, please refer to paragraph 7.1 to 7.7.

5.17 Data on exposure from other potential non-dietary sources (e.g. from consumer products such as cosmetics, from pharmaceuticals) should be provided, where relevant.

Estimate of exposure to substances of safety concern

5.18 Exposure estimates should be provided for substances of safety concern identified in the compositional analysis (e.g. residues, contaminants or degradation products). These substances may be present in the cultured meat due to its source or the manufacturing process, as well as due to its use and storage.

5.19 The same approach as that used for the anticipated intake of the cultured meat should be followed to estimate the exposure to substances of safety concern from the cultured meat for the proposed target population. The maximum amount of these substances expected to occur in the cultured meat (e.g. maximum limit set in the specifications or, in the case that specifications are not established, the maximum level reported among the batch-to-batch analytical data) and the highest estimated daily intake (i.e. 95th percentile) of the cultured meat for the proposed target population should be considered. The exposure to those substances from the background diet should also be considered.

5.20 The exposure to substances of safety concern from the cultured meat (plus from the background diet when relevant) should be compared with HBGVs (e.g. ADI or TDI), when available. When relevant, the potential double accounting of substances of safety concern from the cultured meat and the background diet, especially in those case in which the exposure to substances of safety concern from the diet already exceeds the HBGV, should be considered.

Precautions and restrictions of use

5.21 When proposing precautions (including directions for its

preparation and/or use) and restrictions of use, all available information on safety should be taken into consideration. The population groups (including population groups with certain physiological conditions) which should avoid consumption of the cultured meat should be specified, and the rationale should be included.

Absorption, Distribution, Metabolism and Excretion (ADME)

6.1 Information on the nutritional and toxicological impact of the cultured meat is an important part of the safety assessment, therefore the data on absorption, distribution, metabolism and excretion (ADME) in human and animal should be assessed.

6.2 The chemical, physiochemical and microbiological characteristics of the cultured meat, including its nutritionally and toxicologically relevant components, should be considered. A comprehensive literature review of the existing ADME data on the cultured meat or its relevant components should be conducted, and the collected evidence should be critically appraised.

6.3 In some cases, ADME studies may not be needed, for example, when the cultured meat is composed of substances described to be commonly found in the body or in the diet. However, in the case of nutritionally relevant constituents, ADME assessments should be conducted in accordance with the information provided in paragraph 7.1 to 7.7. The (relative) bioavailability of a nutrient needs to be specifically assessed and quantified when the cultured meat is also a new nutrient source.

6.4 Where there is a potential concern about the protein in the cultured meat, appropriate protein digestibility studies should be performed as part of the weight of evidence approach for the assessment of the nutritional, toxicological and allergenic properties.

6.5 Where the cultured meat consists of polymers > 1000 Da and where evidence is provided that they are not degraded in the GI tract to fragments < 1000 Da, ADME studies may not be needed.

Tiered approach to conducting ADME studies

6.6 ADME studies are to be conducted following a tiered testing approach. Reference for a tiered approach to ADME studies of the cultured meat can be made to OECD Test Guidelines (TG) 417. A description of the data requirements for the different tiers for ADME and the triggers prompting the need to move to a higher tier of data requirements are described below. The need to conduct ADME studies may be waived provided that a scientific rationale is given.

Tier I ADME testing

6.7 Tier I involves assessing all relevant data from the published literature documenting in vitro and in vivo studies on the ADME of the cultured meat or its components and potential metabolites. Chemical and physiochemical data may predict the dissociation characteristics of the cultured meat under GI conditions, which may have an impact on intestinal absorption.

6.8 Tier I also includes the assessment of the comparative metabolism of the cultured meat. This is to establish, where applicable, that the pattern of metabolites formed in human in vitro test systems (as a surrogate of the in vivo situation) is comparable to that in the animal species tested.

6.9 In case there is evidence that the cultured meat per se or its derived components are not absorbed in the small intestine, in vitro (e.g. M-ARCOL, SHIME, Triple co-culture) studies mimicking the human gut and its microbiota dynamics should be conducted to identify or quantify relevant cultured meat-derived metabolites which could potentially be of safety concern.

6.10 If Tier I data do not provide sufficient information for the ADME assessment, or if there are triggers prompting higher tier data requirements, Tier II or Tier III studies are required.

Tier II ADME testing

6.11 The triggers leading to Tier II testing in animals including one or more of the following: (i) indications that the cultured meat or its constituents are absorbed or systemically available; (ii) evidence for the accumulation in the body or formation of metabolites of concern.

6.12 In Tier II, ADME information from both single-dose administration and repeated dose studies (e.g. satellite groups from a sub-chronic toxicity study) in vivo is needed. Tier II ADME information would also be required where e.g. a prolonged half-life or enzyme induction is observed or expected. For the repeated dose ADME study, it is advised to use appropriate samples generated in subchronic toxicity study (OECD TG 408). Guidance for the Tier II ADME assessment is also provided in OECD TG 417.

Tier III ADME testing

6.13 The purpose of Tier III is to generate ADME information on the cultured meat in humans when one or more of the triggers described below is activated. The triggers leading to Tier III testing (ADME studies in humans) include one or more of the following: (i) substantial differences in ADME between different species, (ii) substantial differences in comparative in vitro metabolism studies between the test species and humans (where no suitable animal models exist), (iii) evidence for bioaccumulation of the culture meat, its components or metabolites thereof in the test species.

Specific considerations for cultured meat that are new nutrient sources

6.14 For the cultured meat which is also a new nutrient source, the

bioavailability of the nutrient from the new source needs to be demonstrated. Conclusions on bioavailability should consider the information provided in the section on ADME and the following: (i) For new sources of micronutrients, i.e. vitamins (including metabolites and new vitamers) and minerals, the relative bioavailability of the micronutrient from the new source needs to be quantified, (ii) For nutrients that are not micronutrients, bioavailability needs to be assessed and demonstrated but not quantified.

Nutritional Information

7.1 For the purpose of demonstrating that the cultured meat is not disadvantageous in terms of nutrition under the proposed conditions of use, nutritional information of the product should be studied.

7.2 The information provided should demonstrate that, at the anticipated levels of intake, the introduction of the cultured meat in the diet is not expected to contribute to an excess intake of nutrients or to adversely affect the nutritional status of consumers by increasing the risk of inadequate nutrient intakes.

Excess intake of nutrients

7.3 The cultured meat is considered nutritionally disadvantageous if its consumption, under the proposed conditions of use, could lead to an excess intake of nutrients for one or more population groups, i.e. exceeding the ULs.

Inadequate intakes of essential nutrients

Antinutrient content

7.4 Considering the source of the cultured meat, information on the antinutrient content in the cultured meat as consumed must be provided and compared to the antinutrient content of comparable foods.

Replacement of food(s) in the diet

7.5 When the cultured meat is intended to replace a conventional food, it should be demonstrated that the nutritional composition of the cultured meat does not differ from that of the conventional food in a way that would be nutritionally disadvantageous for consumers under the proposed conditions of use. This includes the application of a novel production process which can affect the nutrient composition of a food. In such cases, the nutrient composition of the conventional counterpart should also be provided, focusing on the nutrient(s) for which it represents a significant source in diets. Indicatively, foods which contain 15% per 100 g or 100 mL (or 7.5% per 100 mL for beverages) of the reference intakes for vitamins and minerals are considered significant sources.

Specific considerations regarding novel protein sources

7.6 The protein quality of the cultured meat must be investigated if the highest mean consumption of the cultured meat under the proposed conditions of use could substantially contribute to the average requirement for protein for one or more population groups. A substantial contribution is defined as at least 15% of the population-specific average requirement for protein.

Additional information

7.7 In specific cases, data from investigations in in vitro, in silico and/or in animal models and/or human studies may be needed to address the interaction of the cultured meat with the diet and nutrients. The necessity for such studies may arise from information on the source, the composition and the production of the cultured meat, from documented experience on the use, preparation and/or handling of the cultured meat and outcomes of ADME, pharmacological, mechanistic, feeding, toxicological and human studies.

Toxicological Information

General considerations

8.1 The purpose of conducting toxicological studies on the cultured meat is to identify and characterise its potential hazards and to support establishing safe intake levels for humans.

8.2 The compositional data of the cultured meat should be considered before designing and conducting toxicological studies. A comprehensive literature review on the toxicological properties of the cultured meat and/or its relevant components should be performed.

8.3 All relevant available knowledge on the cultured meat and/or its relevant components should be thoroughly considered to determine the need for toxicity studies, and if so, the corresponding toxicological testing strategy, a thorough description of which, is to be provided. The important elements to be considered are listed below.

- The source, production process, identity and composition of the cultured meat;
- Available ADME information;
- Available toxicological information (in silico, in vitro, in vivo studies) on the cultured meat, its constituents (including nutrients) or its metabolites;
- Available human studies and case reports; and
- Available relevant information and safety assessments from non-food uses (e.g. chemical, pharmaceuticals, cosmetics).

8.4 When, despite a comprehensive characterisation and literature review, potential data gaps for the hazard identification or hazard characterisation of the cultured meat remain, appropriate toxicological studies should be conducted aiming to fill the gaps. Such studies should be carried out with a representative test material, i.e. the test material should be derived from the production process, be in accordance with the compositional data and meet the proposed specifications.

8.5 In some cases, toxicity tests using the cultured meat as intended to

be placed on the market may lack the necessary sensitivity to identify potential toxicological properties. In such cases, a concentrate or (an) appropriate faction(s) of the cultured meat may be used to increase the sensitivity of a toxicological study. In all cases where the test material is not the cultured meat, the rationale for its use, detailed information on the preparation of the test material and a comprehensive compositional analysis should be provided.

8.6 Toxicological studies should be conducted in accordance with international guidelines (e.g. OECD) and according to the OECD Principles on Good Laboratory Practice (GLP).

8.7 Toxicological data on structurally related substances and their metabolic profiles may be considered for applying a read-across approach. The applicability of read-across to cultured meat may be limited to defined organic chemicals or simple mixtures.

8.8 The threshold of toxicological concern (TTC) approach may be helpful when assessing the risk of substances at low exposure levels (such as impurities, metabolites and degradation products present in (or derived from) the cultured meat) for which toxicity data are not available. It is advised to consult the EFSA Guidance on the use of the TTC approach in food safety assessment.

Tiered approach to conducting toxicological studies

8.9 Appropriate toxicity studies should be conducted following tiered testing approaches both for genotoxicity and repeated dose toxicity. Reference can be made to the EFSA guidance on the scientific requirements for an application for authorisation of a novel food.

Genotoxicity

8.10 The assessment of the genotoxic potential is a basic component of chemical risk assessment in food safety. Accordingly, genotoxicity testing of the cultured meat and its constituents should aim at identifying

substances which could cause genetic damage in humans.

8.11 A tiered approach for the generation and evaluation of data on the genotoxic potential is recommended. Reference on such approach can be made on the EFSA guidance on the scientific requirements for an application for authorisation of a novel food. For cultured meat as whole foods, it may be necessary to focus on specific constituents. As there is currently no generally agreed-upon genotoxicity testing strategy for foods derived from cell culture, case-by-case evaluations are necessary for the genotoxicity testing strategy for cultured meat.

Repeated-dose toxicological studies

Tier I repeated-dose toxicological studies

Subacute toxicity

8.12 Subacute studies (e.g. 14-day) may be conducted providing the basis for the selection of appropriate doses to be used in the subchronic setting. In this case, the full technical report of the dose range-finding study should be submitted.

Subchronic toxicity

8.13 A subchronic (90-day) toxicity study is often needed as part of Tier I. Such a study is usually required when the cultured meat concerns a substance or contains components of unknown toxicity, when there are no HBGVs for the components of the cultured meat, or when an uncharacterised fraction of the cultured meat may cause safety concerns despite thorough compositional analyses. If a subchronic study is not conducted, a well-reasoned justification should be provided.

8.14 Subchronic toxicity studies should normally cover a period of at least 90 days according to OECD TG 408 and a satellite recovery group can be included for follow-up observations. Sound justification should be provided if deviated from the relevant TG.

8.15 For cultured meat as whole foods, specific considerations may be required with regard to dose selection and the avoidance of possible nutritional imbalances due to a high incorporation level of the test item into the animals' feed. For further guidance on the conduction of subchronic oral toxicity studies with whole foods, it is advised to consult the EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed.

8.16 Further reference on the requirements on subchronic toxicity studies can be made to the EFSA guidance on the scientific requirements for an application for authorisation of a novel food.

Tier II repeated-dose toxicological studies

Reproductive, endocrine and developmental toxicity

8.17 Decisions on whether tests for reproductive and developmental toxicity are necessary should take into account the kinetic and toxicity data, including read-across data from structurally related compounds and available data from the literature.

8.18 Any indications of effects on reproductive organs or parameters, as observed in vitro and/or in vivo, may trigger the need for testing for reproductive and developmental toxicity. Potential additional tests include, but not limited to studies covered by OECD TG 414, 416, 421, 422, 426, 440, 441, 455, 456 and 493. Reproductive and developmental toxicity testing may not be required if scientifically justified on a case-by-case basis. Guidance on the triggers for these studies and the details on the implementation on such studies can be found in EFSA guidance on the scientific requirements for an application for authorisation of a novel food.

Other tier II studies

8.19 The need for other studies, e.g. studies on neurotoxicity, cardiovascular effects, immunotoxicity, hypersensitivity and food intolerance, mechanism (mode of action), may be triggered by findings

reported in the literature or in Tier I or II.

Tier III repeated-dose toxicological studies

8.20 Tier III studies comprise toxicological studies of high complexity regarding the duration and the required number of animals.

Extended one-generation reproductive toxicity study (EOGRTS)

8.21 The extended one-generation reproductive toxicity study (EOGRTS), as conducted according to OECD TG 443, is designed to evaluate reproductive and developmental effects which may occur as a result of pre- and postnatal chemical exposure, as well as evaluation of systemic toxicity in pregnant and lactating females and young and adult offspring. The study covers reproductive/developmental toxicity, developmental neurotoxicity and developmental immunotoxicity. Indications of such toxic effects (from the literature, in vitro, in vivo and/or human studies) may trigger the request for an EOGRTS without the need for tier II studies.

Chronic toxicity and carcinogenicity

8.22 Chronic toxicity or carcinogenicity studies are normally not required. However, in exceptional cases, such studies may be needed (e.g. accumulation of the substance, or hyperplasia observed in subchronic toxicity studies) and should follow the respective OECD TG 451, 452 or 453.

Human data

8.23 Human intervention studies, if available, should be provided, regardless of the primary objective of the study, as long as safety aspects were also investigated. Relevant data may also be derived from observational studies.

8.24 In some cases, human studies may be required to establish the safety of the cultured meat, for example, to investigate further potentially

adverse effects observed in toxicological studies or for effects which cannot be investigated in animals (e.g. psychological outcomes, mental health). In those cases, human studies are required to demonstrate that the proposed use of the cultured meat does not raise safety concerns. When performing such safety studies, elements such as study design, sample size, study population (representative of the target population of the cultured meat), study duration, study endpoints etc. of the cultured meat need to be considered carefully.

8.25 The data from intervention studies and observational studies in humans should be organised and considered according to a hierarchy of study designs, and reflecting the relative strength of evidence which may be obtained from different types of studies.

Allergenicity

Cultured meat derived from sources known to trigger allergic reactions in susceptible individuals

9.1 The default assumption is that the allergenic potential of the cultured meat is at least that of the source. The information outlined in points (i)-(iv) should be provided if available in the literature, whereas the evidence requested in the last point is to be generated:

i. Prevalence of the food allergy related to the cultured meat source.
ii. Type and severity of symptoms triggered by the source.
iii. Potency of the source. For example, from the clinical history, the food portion which caused a reaction may be used to calculate the amount of protein able to trigger a reaction (i.e. minimal eliciting doses of total protein in the food triggering allergic reactions in susceptible individuals).
iv. Known clinically relevant allergenic proteins of the source.
v. Detection and identification of the known clinically relevant allergenic proteins in the cultured meat in at least three batches using appropriate immunological or proteomic approaches, methods of

analysis, the LOD of the methods and the complete protocol for protein extraction.

Cultured meat for which the allergenic potential is unknown

9.2 The information required here mainly addresses the allergenic potential of the cultured meat due to potential cross-reactivity and not the potential for de novo sensitisation, for which there are no validated predictive methods currently available.

- A comprehensive literature search and review which cover the cultured meat, its source, used raw materials, other products from the same source and closely related species of the source. It should include all types of studies such as in silico, in vitro, in vivo and human studies including case reports and all types of evidence which could be an indication for the allergenic potential of the cultured meat, such as in vitro and in vivo reactivity, cross-reactivity, elicitation dose, sensitisation and clinical effects, also considering different routes of exposure (e.g. oral intake, skin contact, inhalation).
- Protein digestibility and/or protein stability performed should be also considered in the allergenicity assessment.
- Information on cross-reactive allergenicity which should follow a tiered approach. Testing for cross-allergenicity should be considered only if cross-reactivity of the cultured meat is demonstrated for known allergens and/or known to trigger severe allergic reactions (e.g. anaphylaxis) in sensitive individuals.

<u>Cultured meat as whole foods</u>

9.3 The allergenicity assessment strategy for cultured meat as whole foods should follow a tiered approach.

9.4 In a first step, a phylogenic analysis of the cultured meat source should be performed to gain initial information on the potential for cross-reactive allergenicity.

9.5 On a case-by-case basis and depending on the phylogenetic differences identified, cross-reactivity analysis may not be needed.

9.6 However, if a phylogenetic relationship with known food allergens triggering severe IgE-mediated allergic reactions in sensitive individuals is identified, a sequence analysis of the most representative proteins of the cultured meat should be conducted.

9.7 Depending on the protein similarities identified with known allergens and their associated clinical relevance, the investigation should proceed as outlined in Tier III and Tier IV.

Tier I	Information on the allergenicity of the cultured meat's source organism, based on a comprehensive literature review and its phylogenetic relationship with known allergenic sources.
Tier II	Bioinformatic search for cross-allergenicity, i.e. amino acid sequence (AAS) comparison and complementary approaches.
Tier III[2]	Human serum specific IgE binding assay using immunoassay methods such as ELISA or electrophoresis combined with immunoblotting with serum IgE sera.
Tier IV[3]	Human studies e.g. skin-prick tests and an oral food challenge (preferably double-blind placebo-controlled) in subjects with confirmed food allergy to the known allergen.

Analytical Detection Method

10.1 The methods for detection and quantification of the ingredient or its degradation products (where relevant) in the cultured meat need to be

[2] Required only if bioinformatic analyses indicate potential cross-allergenicity to a known allergen

[3] Required only if IgE binding assays indicate potential cross-allergenicity to a known allergen

robust and applicable for analytical laboratories to determine compliance of any legal limits.

Any Safety Assessment Reports Conducted by Food Safety Authorities in Other Countries / Places

11.1 If the cultured meat has been evaluated by the food safety authorities in other countries or places, the status of the evaluation by each regulatory body (if more than one), should be included as appropriate.

11.2 For evaluations that are under the consideration of other food safety authorities, the proposed conditions of use (if they are different) of the cultured meat, the date of submission, and the recipient regulatory body should be specified.

11.3 For evaluations that have been withdrawn, the conditions of use (if they are different) of the cultured meat which was withdrawn, the date of withdrawal, the reasons for withdrawal and the regulatory body at the time of withdrawal should be indicated.

11.4 For evaluations that have been authorised, the conditions of use (if they are different) of the cultured meat which has been approved, the date of approval and the authorising regulatory body should be indicated. A copy of the scientific opinion of the regulatory body which authorised the cultured meat should be provided.

11.5 For evaluations that have been rejected, specify the date and the reasons of rejection. Indicate the regulatory body which rejected the cultured meat, and if available, a copy of the scientific opinion of the regulatory body which rejected the cultured meat should be recorded.

Any Other Relevant Information to Support the Safety

12.1 Any other relevant information that can support the food safety of the cultured meat should be assessed together with justification of the inclusion criteria.

Concluding remarks

13.1 A concluding remark should include the following information.

- Overall considerations on how the provided body of evidence supports the safety of the cultured meat under the proposed conditions of use
- Where potential health hazards have been identified, they should be discussed in relation to the anticipated intakes of the cultured meat and the proposed target populations
- Considerations of the relevance of toxicologically and nutritionally relevant components (e.g. impurities, by-products, residues, chemical or microbiological contaminants and nutrients) in relation to their estimated intakes, possible background exposure and their health-based guidance values
- Considerations of the results of toxicity studies
- Considerations of any adverse effects identified through the human data
- Considerations of sources of uncertainties

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