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Scientific Opinion on Animal Toxicological Studies for the Safety Assessment of Cultivated Food Products

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Abstract

Background: As the field of cultivated meat and seafood progresses, traditional approaches to risk assessment, particularly whole-food animal feeding studies, require critical evaluation. Animal testing, especially long-term feeding studies, are resource and time-intensive, poses unreliable interpretation, and are ethically contentious. Given the controlled production environment of cultivated foods and the availability of compositional, molecular, and exposure data, this paper examines the applicability of a weight-of-evidence framework as a scientifically robust approach to safety assessment. Scope and approach: The objective is to critically evaluate the scientific justification for *in vivo* toxicological studies in the safety assessment of cultivated food products and examine alternative, non-animal based methods. Key findings and conclusions: Drawing on regulatory precedents, analytical frameworks, and case studies, this review demonstrates that a tiered, weight-of-evidence framework integrating compositional analysis, exposure assessment, and New Approach Methodologies offers a scientifically rigorous and proportionate strategy in comparison to applying uniform requirements for animal studies.

Keywords: cultivated food production; alternative to animal testing; new approach methodologies; repeated-dose toxicity study; regulatory toxicology; food safety

1. Introduction

Cultivated food products are produced by growing animal or plant cells in controlled environments (*in vitro*) rather than through traditional farming methods, with the potential to replicate or improve the sensory and nutritional profile of conventional foods. Given the novel production methods, rigorous scientific evaluation with pre-market authorisation serves as a vital step in safeguarding public health before these products are introduced to the market (Ong et al., 2021).

The regulatory frameworks applicable to cultivated products are highly diverse, with each authority adopting distinct approaches based on regional priorities and scientific considerations. For example, in the European Union, a tiered approach for novel foods emphasises a stepwise evaluation process (EFSA NDA Panel et al., 2024), and the European Food Safety Authority (EFSA) has indicated that animal toxicological studies may not be required depending on the nature of the food (EFSA et al., 2024). In contrast, emerging guidelines for cultivated food products, such as those from South Korea and Thailand, propose the necessity of conducting animal studies such as the repeated-dose

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90-day oral toxicity studies in rodents. Such an approach contrasts markedly against other regulatory jurisdictions in the United States (US), Singapore, Australia/New Zealand, and Israel, where cultivated food products have already been reviewed by regulatory agencies and cleared for consumer sale without such requirements (Hocquette et al., 2025). These differences reflect each authority's discretion in determining the appropriate level of evidence based on the nature and composition of the assessed product. Importantly, requiring default animal trials risks slowing innovation without improving consumer safety, especially in light of international commitments to the 3Rs (replace, reduce, refine).

Safety assessment of cultivated products for human consumption can be achieved through a weight-of-evidence or tiered assessment approach as adopted in jurisdictions such as Europe, US, Australia/New Zealand, and Singapore. These approaches focus on comprehensive compositional analysis, comparison to established food products, and a thorough assessment of the inputs, including culture media components, scaffolds, processing aids, and other materials used during manufacturing, together with assessment of potential contaminants, impurities, and allergenic risks. Such evaluations are similar to frameworks already applied to other novel foods, such as recombinant proteins or oligosaccharides, where safety assessments focus on potential exposure and residual risks from the controlled inputs, rather than the whole food itself (Ong et al., 2025). A review of the available literature and data (identity, composition, origin, historical use, toxicological studies, and intended applications) on cultivated food ingredients and their inputs is a critical first step to determine whether toxicokinetic or *in vivo* toxicological studies are necessary. This is aligned with safety assessments for food ingredients by EFSA or US Food and Drug Administration (FDA) that focus on exposure-based evaluations rather than defaulting to whole-food toxicity studies.

The production of cultivated foods occurs in tightly controlled production processes, where the cells, inputs, and processes are well defined and closely monitored. This level of control allows for comprehensive characterisation of the final product, including its composition and any potential residues from media components, scaffold, or processing aids. Since many of these components are already present in conventional foods or are endogenous to animal cells, their safety can be directly assessed through compositional and exposure-based analyses. The central purpose of animal toxicological studies is to assess potential health risks from whole food. However, when the product's constituents and exposures are already known and well characterised, *in vivo* testing of whole foods becomes unnecessary. While the precautionary principle is relevant, it should not override scientific evidence and modern, non-animal testing methods that can provide more relevant, specific, timely, and humane solutions for safety evaluation.

The objective of this review is to critically evaluate whether *in vivo* toxicological studies are appropriate for the safety assessment of cultivated food products and evaluates how a weight-of-evidence approach, integrating non-animal and alternative testing methods, can provide a robust and proportionate framework for their safety assessment.

2. Realistic Risk Assessment of Cultivated Food Products

Hazard and Risk Framing

A realistic risk assessment of cultivated food products requires distinguishing between hazard and risk. Hazard refers to the intrinsic potential of a substance or process to cause harm, while risk evaluates the likelihood and extent of exposure to that hazard (Barlow et al., 2015). While there are hazards common to both conventional and cultivated products, the focus of this paper is on the novel hazards specific to cultivated products. These must be evaluated based on their likelihood of occurrence and expected consumer exposure. Expert consensus suggests that most hazards in cultivated foods are already well-characterised and are present in conventionally produced food (FAO/WHO, 2023). Risk assessment typically begins with a comparison to conventional foods, which serves as a reference point for composition and safety benchmarks. Selecting an appropriate comparator is important, particularly as single-cell cultivated products differ from more complex

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tissues in conventional meats. Building on this baseline, a weight-of-evidence approach integrates data from existing literature, read-across methods, and biochemical, *in vitro* and *in silico* analyses. This tiered framework ensures that potential hazards are evaluated in proportion to their relevance and exposure, while avoiding unnecessary or duplicative testing.

Comparative Baseline

Conventional products provide a reference framework for cultivated foods across composition, residues, impurities, and nutritional value. Comparative analysis of macronutrients, micronutrients, residues (*e.g.*, culture media inputs, scaffold), allergenicity, and contaminants (*e.g.*, heavy metals, veterinary drug residues, pathogens) help identify differences from conventional products including meat and seafood. This structured comparison distinguishes novel hazards from those that are already well understood, focusing efforts on relevant risks while ensuring alignment with existing food safety paradigms.

Composition

Nutritional composition analysis is an essential part of the safety and regulatory assessment for cultivated products. Differences from conventional meat and seafood are expected, reflecting the differences in cell culture environment. These may be beneficial (*e.g.*, reducing saturated fats), while others may require safety or nutritional evaluation. For example, EU's regulation indicates that novel foods must not be nutritionally disadvantageous to the consumer. Although nutritional equivalence is not always a strict requirement, regulators generally expect manufacturers to characterise compositional deviations, especially if the product is intended to substitute for conventional products. These data inform both safety evaluations and product labelling, ensuring that any compositional differences do not introduce food safety risks and are contextualised within the overall diet. By characterising nutrient content, compositional differences are well understood and appropriately evaluated (UK FSA, 2023). Nutritional differences can be measured directly through established analytical methods, such as proximate analysis, amino acid profiling, fatty acid analysis, micronutrient assays, and *in vitro* digestibility studies, allowing robust comparison with conventional benchmarks.

Macronutrients, including proteins, fats, and carbohydrates, form the first point of comparison. Cultivated products that have a different amino acid composition may affect protein quality and digestibility. These changes are readily evaluated using established biochemical assays (*e.g.*, high performance liquid chromatography, gas chromatography, mass spectrometry, *etc.*) and *in vitro* assays (*e.g.*, simulated digestion). If differences are observed, they can be contextualised against dietary requirements and the overall diet.

Lipid composition is another part of nutritional risk assessment. Key points of evaluation include imbalances in essential fatty acids, potential for lipid oxidation, and trans-fat formation, which are closely linked to increased risk of cardiovascular disease and are subject to regulatory limits (EUR-Lex, 2019; Pipoyan et al., 2021). These hazards are similar to that in conventional meat and seafood. In cultivated systems, changes may occur under suboptimal culture conditions, leading to stress-related by-products, and during shelf-life, where oxidation processes continue to affect lipid quality. Lipid parameters can be systematically evaluated using established analytical methods. Fatty acid profiles can be analysed through gas chromatography to quantify saturated, monounsaturated, polyunsaturated, and trans fats. Lipid oxidation stability can be evaluated by peroxide and anisidine values, thiobarbituric acid reactive substances (TBARS), gas chromatography-mass spectrometry (GC-MS). This structured analytical approach ensures that essential fatty acids are maintained at nutritionally relevant levels, oxidation products remain within acceptable limits, and trans-fat formation is minimised.

Vitamins and minerals may vary between cultivated and conventional foods, depending on the culture medium and metabolic activity of the cells. Standardised micronutrient assays (*e.g.*, HPLC, ICP-MS) allow precise quantification of these differences. From a risk assessment perspective,

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micronutrient composition is interpreted against established dietary reference values and health-based guidance values (*e.g.,* tolerable upper intake levels, recommended dietary allowances). These evaluations allow risk assessors to establish whether deviations are nutritionally meaningful or pose potential safety concerns. As cultivated products are consumed as part of a broader diet, assessments take into account expected intake patterns and contribution to overall nutrient exposure, ensuring cultivated products are not nutritionally disadvantageous while maintaining consumer safety.

Cell Line Characterisation

Cell lines are the foundation for cultivated products, therefore their proper identification and characterisation is critical for safety. General considerations include species identity, cell type confirmation, and stability (UK FSA, 2023). Confirming the cell line originates from the correct species ensures traceability, prevents misidentification or cross-contamination, and provides the foundation for comparison with reference products. Standard authentication approaches, such as DNA barcoding and molecular authentication, are widely applied. Confirmation of the cell type is key to achieving the expected composition of the final product and for normal and consistent behaviour throughout cultivation. These aspects can be evaluated by monitoring morphology, growth kinetics, and cell markers, amongst others. Finally, genetic and phenotypic drift is analysed to ensure that long-term culture does not introduce safety concerns. While most variation is benign and expected, instability may alter gene expression in ways that lead to production or upregulation of hazardous substances, such as allergens. Genetic stability of cell lines can be assessed using non-animal methodologies, such as morphological analysis, karyotyping, and transcriptomic, genomic, or proteomic techniques, as well as monitoring of normal growth characteristics and physiological parameters (de Macedo et al., 2024). These approaches are well-established in pharmaceutical and biotechnological applications for cell line characterisation and quality control, and have been applied to cultivated meat cell lines such as spontaneously immortalised chicken embryonic fibroblasts (Pasitka et al., 2023). Where genetic modification (GM) is used to establish or optimise a cell line, additional characterisation of any introduced or modified sequences is necessary to ensure that no unintended hazards are created. However, such assessments are well established for other food and feed applications, and risk assessment pathways for GM-derived proteins and traits such as read-across approaches (*i.e.,* prediction of the toxicological properties of a 'target' substance using existing data from structurally and/or mechanistically similar 'source' substance), *in vitro* digestion, heat denaturation studies, alongside *in silico* assessments for toxicity and allergenicity are already widely accepted in GM and novel food risk assessments.

Residues

Culture media used in the production of cultivated meat and seafood contains a variety of components. Each component is carefully evaluated to establish their suitability for food use, including the source, composition, impurities, and toxicological profile. Some regulatory guidance exists in evaluating culture media inputs (SFA, 2025), and efforts are underway to develop appropriate frameworks for safety assessment. For example, the Singapore Food Agency (SFA) provides guidance on general information requirements for culture media, and the Safety Assessed Media Ingredient (SAMI) Framework is being developed to identify suitable methods for safety assessment and establish safe-use levels (Ong et al., 2025). While industry and regulatory consensus is still evolving, these efforts aim to support more efficient and fit-for-purpose safety evaluations.

Residual levels of culture media components in harvested cells can be adequately assessed and generally demonstrated to not pose a safety concern provided that all inputs are identified and a sufficient Margin of Exposure (MoE) can be established relative to the relevant reference point, such as history of safe use (*e.g.,* levels in conventional meat), or toxicological values (*e.g.,* no-observed-adverse-effect-levels (NOAEL) from dietary studies). Where a reference point is not available, precautionary and risk-appropriate approaches are required. Steps may include a thorough literature review and read-across from structurally or functionally similar compounds. This helps establish a

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baseline for understanding potential risks associated with the substance, even if a direct toxicological reference is not available. If these approaches are insufficient, the Threshold of Toxicological Concern (TTC) methodology may be applied to derive conservative exposure thresholds under which compounds are assumed to pose negligible risk (Kroes et al., 2005; Munro et al., 2008). Degradation products may also be considered, particularly if the substance has the potential to undergo chemical changes during processing and yield metabolites with different toxicological profiles. New Approach Methodologies (NAMs), such as physiologically based kinetic and toxicodynamic models (*e.g.*, EFSA's TKPlate platform), can simulate realistic exposure scenarios and biological interactions without animal testing (Dorne et al., 2023).

Scaffolds are not always part of cultivated food production, but when used, they can support cell attachment, growth, differentiation, and maturation, especially in structured products. Current regulatory guidance recognises that scaffold evaluation forms part of the overall risk assessment (SFA, 2025). Many scaffolds are made from conventional food materials or biomaterials, such as alginate, gelatin, or plant-based polymers (Park et al., 2025). If scaffolds remain in the final product or degrade into residuals, their safety is evaluated, including potential allergenicity or inflammatory responses (Bomkamp et al., 2022). If scaffolds are intended to be removed, it is critical to ensure that no hazardous components bioaccumulate in the product (Alam et al., 2024). Evaluation methods generally parallel those used for culture media components, including characterisation of composition, impurities, and degradation products. As with other inputs, evaluating the history of safe use or calculating a sufficient MoE is supportive in demonstrating safety.

A potential consideration in cultivated food safety is the production of novel metabolites or small molecules not typically present in conventional meat and seafood. In principle, cellular metabolism could yield unexpected secondary products, and if such compounds were produced at appreciable levels, they might raise toxicological or intolerance concerns. Modern analytical approaches, including metabolomics and whole genome sequencing (WGS) are sensitive tools to characterise the genetic and metabolic profiles of the product (Li et al., 2020). This concern may be largely theoretical for food-relevant species, as there is already a well-established understanding of their genetic and metabolic profiles, with comprehensive resources such as the Livestock Metabolome Database (Goldansaz et al., 2017). This strong baseline understanding means that novel or hazardous metabolites are not expected to arise simply from culturing food-relevant cell types under controlled conditions. Any detected compounds may be contextualised against existing dietary exposure data, toxicological reference values, or allergenicity data.

Impurities

Conventional meat and seafood originates from whole animals, which may harbour toxins or pathogens. In contrast, cultivated meat and seafood is produced in controlled environments, where factors such as contamination and disease transmission are significantly reduced. As a result, the overall risk profile is lower compared to conventional food sources, especially when robust safety management measures are in place. Cell cultivation occurs under stringent aseptic conditions, while post-harvest product formulation occurs in standard food manufacturing environments where conventional hygiene risks, such as personnel, equipment, and the processing environment become relevant (Powell et al., 2025). These contaminants can be monitored and evaluated using established food microbiological methods.

Potential contaminants from the inputs or process, such as antibiotics, fungicides, endotoxins, and heavy metals, may remain in the final product as residues. These are mitigated, removed, or reduced to safe levels consistent with established toxicological thresholds. During cultivation, certain substances may bioaccumulate in the cells. Identification of potential contaminants and impurities, and targeted testing for these residues is a routine part of safety assessment of cultivated products.

Allergenicity

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Allergenicity assessment focuses on potential allergens from the inputs (*e.g.,* media, scaffolds) and intrinsic allergenic potential of the cells. For inputs, the key concern is whether residues of allergenic source materials (*e.g.,* wheat, soy) persist in the final product at levels that could trigger reactions in sensitive individuals. For cells, assessment focuses on the source species and potential to express novel proteins with allergenic properties. Evaluating the allergenicity of protein-based foods presents challenges in all novel foods since thresholds of acceptable allergenic risk are not universally established (Fernandez et al., 2021; Crevel et al., 2024). In cases where the cell source is from a species already recognised as allergenic (*e.g.,* shellfish), the expectation is that the cultivated product would carry similar allergenic potential and must be labelled accordingly. Currently, allergen assessment approaches rely on a weight-of-evidence approach, combining *in silico* bioinformatic sequence comparisons with known allergens, *in vitro* assays such as simulated digestion to evaluate protein digestibility or IgE-binding studies, and where possible, read-across from conventional comparators (Mills et al., 2024). These methods help identify proteins with structural or functional similarities to established allergens and assess whether they are likely to retain allergenic potential.

Summary

All these potential hazards that may be considered novel in cultivated food production can be appropriately evaluated using biochemical assays, *in vitro* testing, or *in silico* models that have a widely established history of use in the assessment of food safety for regulatory approvals. These established methods and models thereby limit the need for food safety authorities to require animal testing to demonstrate the safety of cultivated food products for consumption, as is the case for a range of other novel food products that have entered the food supply such as fungal cell mass in the US (*e.g.,* GRAS 1117); or the examples described in Section 3.2.2 in EU.

3. Rationale for Employing Alternate Approaches to Animal Feeding Studies

As the field of cultivated food progresses, traditional approaches to risk assessment, particularly whole-food animal feeding studies, require critical evaluation. Animal testing, especially long-term feeding studies, are resource and time-intensive, poses unreliable interpretation, and are ethically contentious. Moreover, given the controlled production process and ability to evaluate compositional, toxicological, and exposure data within a weight-of-evidence framework, *in vivo* studies are unlikely to be necessary for most cultivated products. Proportionate and science-based assessment strategies can rely on compositional comparability and a targeted evaluation of inputs and residues. This aligns with the established regulatory approach for GM plants and precision fermentation products, where safety assessments focus on compositional analysis, molecular characterisation, toxicological reference values, and exposure estimates, rather than routine *in vivo* testing. A tiered weight-of-evidence framework provides confidence that when the safety profile of the inputs and residues are understood and exposure levels are well characterised, robust safety assessments can be achieved without reliance on whole-food animal feeding trials.

3.1. Limitations of Whole-Food Animal Feeding Studies

Whole-food animal feeding studies face some scientific and practical limitations. Low interspecies concordance may reduce the predictive value of animal models for human health particularly when the absorption, distribution, metabolism and excretion profile of inputs is unknown (Van Norman, 2019). Incorporating whole foods into animal diets may require processing, such as freeze-drying and powdering to ensure consistent dosing. This processing can alter a food's nutritional and chemical properties, affecting the study's outcomes. Animal testing requires administering unrealistically high doses to establish a toxicological reference point. For whole foods, this may result in nutritional imbalances or adverse effects unrelated to realistic human exposure (*e.g.,* kidney damage or digestive disturbances) (Bartholomaeus et al., 2013). Importantly, rodents are not adapted to a fully meat-based diet (EFSA Scientific Committee, 2011), feeding them high levels of

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animal protein can result in adverse physiological responses unrelated to human dietary patterns. For protein-rich products, amino acid imbalances may occur, confounding interpretation of the results. Additionally, whole-food animal studies often lack the sensitivity to detect small but relevant toxicological effects, and attribute an observed effect to a specific constituent (Bartholomaeus et al., 2013). As such, these studies rarely yield a meaningful toxicological reference point, and the derived MoE may not be informative for human risk assessment.

3.2. International Precedents

3.2.1. Existing Cultivated Food Approvals

Regulatory precedents in Singapore, US, Israel, and Australia/New Zealand did not require whole-food animal studies to demonstrate the safety of cultivated products. Instead, risk assessments emphasised compositional comparability, genetic and product stability, and analysis of inputs and residues, relying on biochemical, *in silico* and *in vitro* tests. These approaches reflect proportionate risk assessment strategies aligned with established food safety science.

3.2.2. European Union

Although no precedents are currently available for EFSA opinions on cultivated products from animal cells, EFSA has adopted a tiered approach to evaluating the safety of novel foods, in which the need for animal testing is determined based on the level of concern associated with the product's composition and any unknown risks. Subchronic (90-day) rodent studies are often required for products with components of unknown toxicity, where there are no health-based guidance values, or contain uncharacterised fractions that could pose safety concerns (EFSA NDA Panel et al., 2024). Only 50 out of 190 novel food opinions published by EFSA (till 15th October 2024) derived its reference point from toxicological studies (not always a 90-day study) (Rivero-Pino, 2025). For example, in the assessment of apple fruit cell culture biomass, the provided subchronic toxicity study was not needed to establish the safety under the proposed conditions of use (EFSA NDA Panel et al., 2023). In the remaining opinions, no additional toxicological studies were required, or the available toxicological data was not suitable or relevant to derive a toxicological reference point. In the case of human-identical milk oligosaccharides (HMOs), safety assessment focus on comparing the intake of the HMO/novel food under proposed use conditions with natural human milk intake by breastfed infants. Animal studies were impractical due to its viscosity and laxative effects at high doses, and subchronic toxicity studies were limited by the low MoEs, thus safety assessment relied on comparisons with natural human milk intake (EFSA et al., 2024).

3.2.3. China

Animal feeding studies are generally required for novel food products in China. According to two national standards issued by the National Health Commission, a tiered approach towards animal-based toxicological tests are used (NHC, 2014; NHC, 2021). As a common practice, China also looks at the clearance status of a novel food product in other major jurisdictions, such as the US and EU for considering its regulatory approvals. Recently, several HMO products have been approved as novel foods in China, but it is unclear whether the requirement for animal feeding studies was waived in such cases.

3.2.4. Thailand

Animal toxicity tests are specifically required within Thailand's cultivated meat regulatory guidelines (BIOTEC, 2024). However, there is ongoing discussion between the industry and the Thai FDA to allow for alternative strategies that can satisfy the need to demonstrate safety instead of using subchronic whole-food animal studies.

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3.2.5. South Korea

South Korea currently requires subchronic feeding studies for all novel food applications, including cultivated food products (MFDS, 2024). It remains unclear whether a repeat of *in vivo* feeding trials would be required for the process of formulation changes.

3.3. Innovation and Commercial Implementation Barriers

The high cost and long timeline of whole-food animal studies pose significant practical barriers for startups and small companies in an emerging field that prioritises the ethical treatment and well-being of animals. A 90-day oral toxicity test in rodents is estimated at USD $213,400 (US EPA, 2025) and can take over 12 months to complete. If the safety of cultivated products is based on weight-of-evidence and exposure assessments of all inputs (See Section 2) rather than animal testing, it allows for greater flexibility in product evaluation which would encourage applicants to file dossiers in those jurisdictions. For example, changes to individual culture media components can be assessed independently without the need for a new animal study, provided the data are sufficient to ensure safety. If each change were to trigger new animal studies, cultivated food producers would not have sufficient resources to meet these requirements. A recent example is an approved cultivated Coho salmon, which underwent multiple modifications to cell culture media, bioprocess steps, and end product changes over the course of the assessment by the US FDA (US FDA, 2025). The US FDA required new compositional, toxicological, and microbiological testing prior to a no questions letter being issued, but did not mandate an animal study, as the changes did not raise new safety concerns. This demonstrates how tiered, weight-of-evidence approaches provide both flexibility and scientific robustness, without the disproportionate burden of repeated animal studies.

3.4. Specific Cases Where In Vivo Testing May Be Warranted

While most hazards can be addressed using non-animal methods, *in vivo* studies may be occasionally justified under specified conditions. For example, animal testing may be considered when there is a novel media input or expressed protein (*e.g.*, expressed as a result of genetic modification) that has no history of safe use, no available toxicological information, and significant expected exposure. In such cases, an *in vivo* study of that component may be needed to derive a reference point. However, animal studies should be viewed as the final tier in a modern, weight-of-evidence safety assessment for cultivated products. The decision to proceed with animal studies must weigh the potential scientific value of the information gained against the likelihood of producing inconclusive or non-translatable results. A tiered, weight-of-evidence strategy that integrates data from existing sources, structural analogues, and validated non-animal methods should therefore remain as the foundation of cultivated food products safety assessment. Regulators are increasingly encouraged to accept these NAMs as scientifically valid alternatives, particularly where animal testing offers limited additional value or raises ethical concerns.

4. Design of Feeding Trials: Case Study

The precautionary principle was invoked by Italy in relation to the safety of cultivated food when it enacted a ban in 2023, and by a note issued by delegates of some EU member states to the EU Council in 2024 (Monaco, 2025), citing that cultivated foods may pose unknown risks to consumer health and called for *in vivo* toxicological testing. However, the absence of published research presents inadequate grounds to invoke the precautionary principle (Rubino & Dal Pozzo, 2024).

To inform this discussion, an unpublished 90-day repeated oral dose toxicity study, conducted by a third-party Good Laboratory Practice (GLP)-accredited laboratory is summarised here. Test abides by the OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17 as revised in 1997) and OECD Guideline for the Testing of Chemicals No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents (Adopted: Jun 25, 2018). The study, provided by a cultivated meat company on condition of anonymity, is included to enrich public knowledge on this topic.

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The study (Table 1) evaluated potential target organ toxicity and established a NOAEL following repeated oral administration of the cultivated cells in Sprague-Dawley rats for 90 days. An additional 28-day recovery period was included to assess reversibility or persistence of any treatment-related effects. Rats had free access to food and water throughout the study. The test material, a diluted cell resuspension in phosphate-buffered saline, was administered orally once daily at 1250, 2500, and 5000 mg/kg body weight/day for 91 consecutive days by gastric intubation using a disposable syringe fitted with an intubation tube.

No toxicologically remarkable or statistically significant changes were observed at any dose. The NOAEL was therefore established to be 5,000 mg/kg bw/day, both in males and females.

Table 1. Summary of the doses, number of animals used, tests conducted and results.

Treatment Dose (mg cells/kg Bodyweight)	Sample Size	Summary of Clinical, Toxicological, and Histopathological Tests Conducted	Results
1,250 2,500 5,000 Control (0.9% saline)	N = 10 males and 10 females in each 90-day repeated dose treatment and 5 males and 5 females in each treatment 28-day recovery group.	Recorded information include clinical signs, detailed observation, body weight, food consumption measurement, drinking water consumption measurement, functional observation battery, ophthalmological examination, urinalysis, hormone test, hematology, blood biochemistry test and sperm test, vaginal smear test, organ weight measurement, necropsy and histopathology.	No toxicologically remarkable or statistically significant changes were observed for any test.

Rat dietary toxicological studies are typically conducted at relatively high dose levels – often up to a practical limit of 2,000 mg/kg bw/day. In the present study, a NOAEL of 5,000 mg/kg bw/day was established, well above the conventional upper range. When extrapolating from rats to humans, regulatory norms typically apply a 100-fold uncertainty factor (10x for interspecies and 10x for intraspecies variability) to account for potential differences in toxicokinetics and human sensitivity (FAO/WHO, 2008). Applying this standard factor to the 5,000 mg/kg bw/day NOAEL yields a human equivalent safe intake of 50 mg/kg bw/day, or roughly 3 g/day for a 60 kg adult. This example illustrates that even when no adverse effects occur at very high doses in animal studies, regulatory safety assessment practices, designed to ensure wide margins of protection, can generate conservatively low theoretical intake limits that do not necessarily reflect realistic dietary exposure for whole foods.

5. Scientific Validity and Regulatory Acceptance of NAMs in Risk Assessment

NAMs are alternatives to animal testing for next-generation risk assessment of chemical and food safety. Their adoption in regulatory toxicology serves to meet the 3Rs (replace, reduce, refine)

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of animal experimentation, while also generating more mechanistic information than traditional animal studies (Manful et al., 2023). NAMs encompass *in vitro*, *ex vivo*, *in chemico* and *in silico* methods, such as cell-based high-throughput screening assays, microphysiological systems (organ-on-chip), organoids, multiomics profiling, physiologically-based kinetic (PBK) models, quantitative structure-activity relationship (QSAR) methods and read-across (Schmeisser et al., 2023).

Case study: Cosmetics

Perhaps the most successful application of NAMs to reduce animal testing is the safety assessment of cosmetics following progressive bans on animal testing beginning in the UK in 1998 (Sewell et al., 2024). Single *in vitro* assays were insufficient to replicate *in vivo* effects, so data integration from several assays with complementary endpoints, termed Defined Approaches, were formalised into OECD Testing Guidelines to assess cosmetic ingredients (Schmeisser et al., 2023; Sewell et al., 2024). These efforts demonstrated that NAMs can successfully replace animal tests without compromising safety and serve as a model for other sectors.

Application for Food

Although NAMs are still being validated to assess systemic effects of complex food matrices, they have already been applied for cultivated food products as part of a weight-of-evidence approach. Examples include the use of read-across data to assess specific media substances, and genomics and transcriptomics to assess the stability of cell lines (US FDA, 2021). The *in vitro* Mammalian Cell Micronucleus Test (OECD TG 487) and Mammalian Chromosomal Aberration Test (OECD TG 473) are also commonly integrated into the first tier of toxicological assessment in several food risk assessment guidelines for novel foods (EFSA NDA Panel et al., 2024; SFA, 2025). Emerging tools such as the ToxTracker assay, a fluorescent reporter cell line used to classify genotoxic and non-genotoxic compounds, and to ascertain their mechanism of genotoxicity, are undergoing RING trials and OECD validation. This assay investigates several mechanisms of genotoxicity and can potentially replace the need to conduct multiple genotoxicity tests (Hendriks et al., 2024). Results of validation studies for 3D organoids and organ-on-chip systems, currently carried out on pharmaceutical or environmental chemicals, can also inform similar validation studies for food-relevant NAMs. These applications highlight the growing use of NAMs in food safety assessment, building on longstanding experience with their use in other domains.

6. Research Gaps and Perspectives

Despite significant progress in cultivated food development, important research gaps remain, particularly in the availability of publicly accessible information and validated analytical or toxicological assays needed for reliable and efficient toxicological evaluation.

While analytical methods for detecting and quantifying production inputs such as growth factors, antifoams, and complex mixtures are available, they are not yet standardised or routinely available. As a result, companies often need to develop and validate their own methods, which can be resource-intensive and costly.

The replacement of fetal bovine serum with other complex mixtures or chemically defined media addresses contamination, variability, and ethical concerns (Lee et al., 2022), but novel formulations must still undergo systematic toxicological evaluation. These studies confirm that alternative media components do not introduce new hazards, ensuring safety and regulatory compliance.

Allergenicity and immunogenicity of novel proteins remain areas requiring targeted investigation. Integrating multi-omics data and advanced biomarkers could support predictive safety assessments.

Another key challenge lies in process variability. Differences in cell lines, culture media, and bioreactor conditions can influence the composition and safety profile of the final product. Research

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is needed to identify which parameters most significantly impact safety and to develop biomarkers or chemical fingerprints that can monitor consistency (Allan et al., 2019; Levenberg et al., 2020).

Finally, while NAMs offer promising tools to predict hazards and prioritise testing, further research is needed to validate these approaches for cultivated food products. Demonstrating their reliability and relevance, and achieving regulatory acceptance, will be key to enabling these approaches in replacing animal-based toxicological testing. Likewise, their progressive integration into risk assessment frameworks is also essential in building regulators' confidence, and to ensure that regulatory science evolves alongside technological innovation.

7. Conclusions

Ensuring the safety of cultivated food products requires approaches that are both scientifically robust and proportionate to their potential risks. A tiered, weight-of-evidence framework integrating compositional analysis, exposure assessment, and NAMs offers a more appropriate strategy than applying uniform requirements for whole-food animal studies. Within this framework, conventional *in vivo* toxicity testing remains available but should only be reserved for cases where uncertainties cannot be resolved through existing knowledge, NAMs, or threshold-based approaches like the TTC.

At present, the 90-day oral toxicity study provides limited added value for whole cultivated food products, as their compositional complexity makes it difficult to attribute observed effects to specific constituents, which may lead to costly, yet unreliable interpretations for food safety purposes as well as commercially impractical daily intake limits as highlighted in Section 4. This issue is compounded by the fact that whole-food animal studies rarely achieve exposure levels that can be meaningfully extrapolated to human dietary intake. More informative insights can be gained through targeted assessment of inputs, contaminants, or process-related substances, supported by *in vitro* and *in silico* approaches. These methods also enable more realistic estimations of MoE and align safety testing with actual human consumption patterns.

Looking ahead, the progressive validation and acceptance of weight-of-evidence approaches provide a clear pathway towards demonstrating the safety of cultivated food products. Such an approach would not only overcome the limitations of animal models in predicting human health effects but also enable more efficient, innovation friendly, ethically responsible, and transparent safety evaluations for cultivated food products.

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