Technical guidance to applicants for the authorisation of Precision Bred Organisms for food and feed

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Purpose

This guidance describes the scientific considerations required for evaluating the safety and nutritional aspects of Precision Bred Organisms (PBOs) when seeking marketing authorisation of PBOs for use in the production of food and feed.

Specifically, to support applicants in understanding:

- How to embed safety management in their process;
- How to perform a safety assessment to determine whether a PBO poses any safety concerns or could be nutritionally disadvantageous;
- Whether to apply under Regulation 20 (when a Tier 1 safety assessment completed by the applicant is sufficient) or Regulation 22 (when an additional Tier 2 safety assessment completed by the FSA is required) of the Genetic Technology (Precision Breeding) Regulations 2025 [cross-reference when available];
- Which information to submit to support an application under Regulations 20 or 22;
- Additional data which may be required for Tier 2 safety assessment and Regulation 22 applications.

This guidance is to be used in conjunction with the Applicant Guidance [link when available]. References to food/feed regulations and obligations under General Food Law are included where they support comprehension. However, this guidance does not constitute a guide to General Food Law, nor does it replace applicants' existing obligations to comply with General Food Law and any other applicable food/feed law.

Summary

This guidance document details the scientific safety assessment process which applicants should undertake in respect of precision bred organisms (PBOs) used to produce food and feed.

There are two routes to apply for a food and feed marketing authorisation which are explained in Regulation 20 and Regulation 22 of the Genetic Technology (Precision Breeding) Regulations 2025 [cross-reference when available]. To determine whether the criteria for an application under Regulation 20 have been met, all applicants must conduct a 'Tier 1' safety assessment of their PBO. Where the criteria in Regulation 20 are not met (i.e., where potential quality or safety concerns are identified), or where there is uncertainty as a result of the Tier 1 safety assessment, a Regulation 22 application should be made for an additional 'Tier 2' safety assessment by the FSA.

Applicants should follow this guidance document to ensure that an appropriate Tier 1 safety assessment is performed and that they apply under the correct regulatory route. Applicants must satisfy their legal obligations as stated in the Genetic Technology (Precision Breeding) Regulations 2025 [cross-reference when available]; where applicants take the steps which this guidance indicates "must" be completed this will maximise the prospect of obtaining a food and feed marketing authorisation in respect of the PBO.

The safety assessment process reviews potential safety concerns regarding food/feed and nutritional quality. It details the types of evidence applicants are to use, and may be asked to provide, when seeking food or feed marketing authorisation for PBOs. The FSA's legal objectives are to protect public health from risks arising from the consumption of food and generally to protect the interests of consumers in relation to food and feed. Therefore, applicants wishing to bring PBOs to market must assess the potential effect(s) of the introduced genetic change to food/feed safety or nutritional quality.

The purpose of the guidance, provided in four parts, is to:

- Outline the scope and the technical aspects of the safety assessment of PBOs including general, compositional and specific considerations for all applications (Part 1).
- Guide applicants through the Tier 1 safety assessment, and to determine whether a Tier 1 safety assessment is sufficient and therefore an application may be made under Regulation 20. Where a Tier 2 FSA safety assessment is required, a Regulation 22 application is required (Part 2).
- Identify the information required for applications under both a Regulation 20 or Regulation 22 (Part 3).
- Detail what additional information must be initially provided when applying under Regulation 22 for a Tier 2 FSA safety assessment, as well as what may be requested during the FSA safety assessment (Part 4).

Applicant-led Tier 1 safety-assessment - Applicants are to perform a safety assessment following this guidance to identify any potential safety concern(s) associated with their PBO. PBOs may possess characteristics that warrant provision of additional information on molecular and/or compositional analyses to permit effective safety assessment.

A step-by-step process is outlined in this guidance document to understand and determine the tiered safety assessment requirements, which is also summarised using flow charts. On completion of this process, **applicants** will determine whether they consider that a Tier 1 safety assessment is sufficient (no additional safety assessment by the FSA), or a Tier 2 safety assessment is required (additional safety assessment by the FSA) for their PBO.

Information required for all applications - The evidence considered by applicants during Tier 1 safety assessment focuses on ensuring compliance with relevant requirements of assimilated Regulation (EC) 178/2002, 'General Food Law'; by following the guidance, applicants are likely to be able to better demonstrate that they have complied with these requirements. For this, the PBO must be considered in comparison to a suitable comparator (see Section 4). Applicants must consider the specifics of the genetic change and the potential for significant impacts on composition: specifically nutrition, toxicity, and allergenicity. Significant impacts to composition are those changes which are biologically relevant to safety or nutritional quality, that are outside the ranges found in traditionally bred comparators that have a History of Safe Food Use (HSFU), or Prior Feed Consumption (PFC) (see Definitions) in the UK or EU, or outside the ranges found in reference food composition datasets. Any non-compositional concerns should be considered under “Other Safety Concerns”.

Information required specifically for Tier 2 FSA safety assessment and Regulation 22 applications - Where composition impacting safety/nutritional quality is significantly altered, applicants must submit a Regulation 22 application for a Tier 2 FSA safety assessment on the specific concern(s). If applicants are unsure that their PBO meets the criteria for a Regulation 20 application, then a Regulation 22 application must be made. In some cases, a Tier 1 safety assessment may identify safety concerns under multiple criteria (e.g. toxicology, allergenicity and nutrition). Regulation 22 applications may require additional data to provide evidence to support an FSA safety assessment. The FSA will evaluate the requirement for further safety data on a case-by-case basis.

A Regulation 22 application may also be needed due to specific restrictions of use, for example, organisms requiring new conditions of use or if the progenitor does not have a HSFU (something that would be considered a “novel food” if it was not a PBO or produced from a PBO). Examples are provided throughout the guidance, though they are not exhaustive of the types of PBO which may be produced.

Data provision to FSA – This guidance document provides specific details on the information which must be provided to the FSA when seeking a Regulation 20 or a Regulation 22 marketing authorisation. The FSA requires a defined data submission for all applications. This consists of demonstration that appropriate evidence on safety of the PBO has been considered, with a summary of the relevant data and conclusions reached by applicants. A verification process will apply to all Regulation 20 applications submitted. This is detailed in the Applicant Guidance [link when available]. For Regulation 20 applications, it is not necessary to provide the full details of all the information and evidence considered during the applicant’s safety assessment, though the FSA may in some circumstances request further details as part of the verification process. For Regulation 22 applications, additional evidence and detail is required, which will need to be provided for FSA safety review. Data that was

used by applicants for safety assessment may be requested by the FSA for Tier 2 safety assessment.

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Part 1 – General Introduction

1. General Considerations for all applications

Precision breeding (PB) describes a range of breeding technologies, such as gene editing, that enable DNA to be edited efficiently and precisely. A "Precision Bred Organism" is defined in section 1 of the Genetic Technology (Precision Breeding) Act 2023. Only organisms containing genomic changes equivalent to those which could be produced through traditional breeding (TB) are recognised as PBOs. Therefore, any potential safety concerns are expected to be no different from those found in organisms obtained through TB. With any breeding process, anticipated effects on the phenotype of the organism should be considered. As with TB, there is the potential to create safety risks for consumers of PBOs, and the likelihood of this must be considered. The generation of PBOs is new and rapidly evolving, and any process and guidance must support the appropriate level of safety assessment required to ensure that potential safety risks are identified, assessed, and managed appropriately by industry.

Applicants are expected to embed safety management into their process, with due consideration for food/feed safety and nutritional quality: applicants will safety assess their PBO with consideration of nutritional value, toxicants, and allergens, in addition to novelty and any other safety concerns which may also lead to adverse health impacts. This guidance helps applicants complete a Tier 1 safety assessment. The conclusions of the Tier 1 safety assessment determine whether further assessment is required by the FSA ('Tier 2 safety assessment'). If it is required, then an application under Regulation 22 must be submitted. The safety profiles of some phenotypes are predictable, based on a similar comparator, where the level of risk is known and has to date been accepted (Tier 1 safety assessment is conclusive). Conversely, some phenotypes will require a higher level of consideration due to existing evidence or significant uncertainties concerning the data that is available to assure their safety (Tier 2 FSA safety assessment is needed).

Unless otherwise specified, Regulations and Schedules referred to in this document are Regulations and Schedules in the Genetic Technology (Precision Breeding) Regulations 2025 [cross-reference when available].

Applicants must ensure they are using the latest version of the technical guidance found on the FSA webpage [link to applicant webpage].

2. Scope

This guidance document should be followed by all applicants seeking a PB food or feed marketing authorisation to ensure an appropriate Tier 1 safety assessment is completed, and maximise the prospect of authorisation. For an FSA food and feed marketing authorisation application, the phenotype of a PBO resulting from the introduced genetic change must be assessed. This includes both the intended phenotype and any reasonably anticipated effects. Similar intended phenotypes may be achieved through different biological mechanisms, resulting in differing potential safety concerns. Therefore, the specific genetic change must also be considered.

This guidance applies to precision bred plants (land plants (Chloroplastida) and certain precision bred algae (seaweeds belonging to the Phaeophyceae, red and green algae as well as some eucaryotic microalgae belonging to the Archaeplastida)) for which a precision bred confirmation is in force, as detailed in the FSA Applicant Guidance. PBO confirmations are issued by the Department for Food, Environment and Rural Affairs (Defra) in accordance with section 8 of the Genetic Technology (Precision Breeding) Act 2023 upon the advice of its Advisory Committee on Releases to the Environment (ACRE).

This guidance **does not apply to**:

- Genetically modified **microorganisms**, including Prokaryotic and some Eukaryotic microalgae, which continue to be regulated under Assimilated Regulation (EC) 1829/2003;
- PBOs which are **animals;** separate guidance will be published should PB animal organisms be added to this regulatory framework in the future.

3. Overview of the tiered safety assessment process

Following a precision bred confirmation from the Secretary of State [link website when available], applicants must complete the safety assessment process outlined in Figure 1 before making an application to the FSA via the correct regulatory route. There are two routes to apply for a food and feed marketing authorisation which are explained in Regulation 20 and Regulation 22 of the Precision Breeding Regulations. To determine whether the criteria for an application under Regulation 20 have been met, all applicants must conduct a 'Tier 1' safety assessment of their PBO. Where the criteria in Regulation 20 are not met (i.e., where potential safety concerns are identified), or where there is uncertainty as a result of the Tier 1 safety assessment, a Regulation 22 application should be made for a 'Tier 2' FSA safety assessment. The steps for submission to the FSA are as follows:

- Tier 1 safety assessment: Part 2 of this guidance leads applicants through the Tier 1 safety assessment and allows them to determine whether a Tier 2 FSA safety assessment is required.
- Information to include in all applications: Part 3 identifies the mandatory information from the Tier 1 safety assessment to be included in applications under both Regulation 20 and Regulation 22.
- Part 4 describes the additional information required to support a Tier 2 FSA safety assessment of applications made under Regulation 22.

Applicants must first characterise the identity of their PBO. The species and the alteration made to the genetic material are essential to understanding the effect of the genetic change. This includes a sufficiently detailed description of the genetic change(s) to evaluate the potential impact of the genetic alteration on the safety and nutritional quality of food and feed (see Section 16). This information must then be used to answer the safety assessment questions (see Section 8).

Five criteria are described in the Regulations 20 (1) (b) and (c), relating to: Novelty; Nutrition; Toxicity; Allergenicity; and Other Safety Concerns. A series of assessment questions are provided to guide the Tier 1 safey assessment for each of these five criteria (Figure 1). The safety assessment questions focus on the immediate phenotypic consequences resulting from the genetic change, taking into account the nature of the genetic change. However, intended genetic changes introduced through the application of modern biotechnology may also cause unintended characteristics in plants. Therefore, during the Tier 1 safety assessment, applicants must consider whether genetic changes may reasonably be anticipated (see Definitions) to unintentionally increase levels of potentially harmful components, or change in nutritional quality (Nutrition). Once the Tier 1 safety assessment is complete, applicants must submit an initial data submission including the mandatory information via the appropriate application route detailing their conclusions.

Where all safety considerations have been addressed, and sufficient information is provided on all criteria to confirm that there are no safety concerns, no further safety assessment is required.

PBOs require a Tier 2 FSA safety assessment where food safety concerns are identified, where the conclusion of any of the criteria cannot be sufficiently evidenced, or where applicants are uncertain about a conclusion concerning any of the criteria. The FSA may require further data to be submitted on a case-by-case basis to address any specific concerns identified and to undertake a safety assessment.

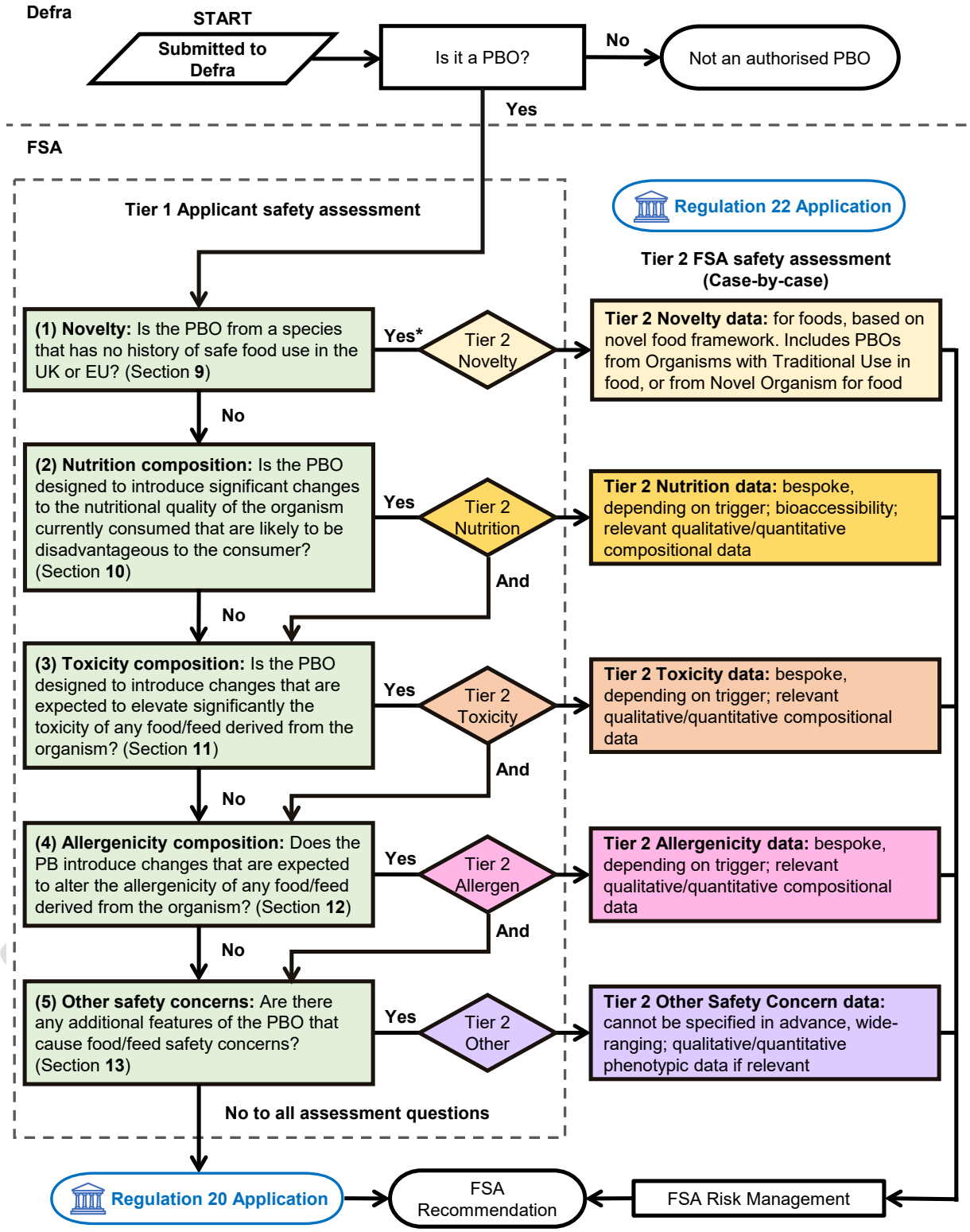

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Figure 1.

Overview of the tiered safety assessment of PBOs for food and feed use. Every Tier 1 safety assessment question (green) must be answered sequentially. For PBOs from organisms with no history of safe food use (*), questions (2), (3), and (4) must also be answered for feed use, and question (5) for both food and feed use; for other PBOs, where a PBO meets any criteria for Tier 2 safety assessment, the remaining safety assessment questions must also be answered. Following completion of Tier 1 safety assessment by the applicant, if the answer to all of the safety assessment questions is 'no', PB food or feed marketing authorisation must be sought

via a Regulation 20 application. Where the answer to any question is 'yes', an FSA Tier 2 safety assessment is required for the corresponding criterion and a Regulation 22 application must be made. For a definition of significance, see Definitions.

Applicants are advised that the recommendations in this guidance must not be regarded as a finite checklist. Alternative approaches are suitable provided they are scientifically justified, generate reliable and conclusive data, and satisfy the applicable status and regulations. **The key is to assure the FSA of the safety of a PBO by providing brief conclusions on the safety of the PBO with respect to each assessment criteria, justified with a summary of the appropriate scientific evidence utilised.**

Applicants are responsible for the accuracy and quality of the data and conclusions provided. **A structured explanatory narrative should present the information in the application**. Provision of a clear and detailed narrative outlining how the data supports the conclusions made on the safety of a PBO will allow the FSA to minimise any delays in processing the application, and will aid the Tier 2 FSA safety assessment of Regulation 22 applications. The FSA retains the power to request or examine further data and may seek more information where potential risks are identified, or further clarity is required.

The FSA will verify all Regulation 20 applications as described in the Applicant Guidance [cross-reference when available] to ensure all the necessary information has been provided as required. Applicants must understand the properties of the PBO requiring a food or feed marketing authorisation in order to assess and conclude on the safety of the PBO. Applicants must clearly communicate any conditions of authorisation corresponding to a PBO in its onwards supply/distribution.

4. Comparators

Applicants must demonstrate whether any compositional change relevant to food/feed use is significant in order to determine whether the criteria set out in Regulations 20 (1) (c) (i) and (ii) are met. During the applicant's safety assessment of nutrition, toxicity and allergenicity, a suitable comparator must be used to determine if a change to nutrition or toxicity is significant and whether the genetic changes introduced by PB are expected to alter the allergenicity of food or feed produced from it - **significant impacts to composition are those changes which are biologically relevant to safety or nutritional quality, that are outside the ranges found in TB comparators that have a HSFU/PFC in the UK or EU, or are outside the ranges found in reference food composition datasets**. This will be used to determine whether a PBO requires a Tier 1 or a Tier 2 safety assessment.

Applicants must exercise their scientific judgement in selecting suitable published food composition datasets (for example, McCance and Widdowson, 2021), or suitable

reference varieties. Reference varieties used for comparative analysis are referred to as comparators.

Applicants may select more than one reference dataset or comparator to demonstrate that a compositional change is within the range of what already exists in food or feed for that species.

All comparators must be selected from non-PBO reference varieties with a HSFU and a composition representative of those varieties normally consumed in the EU or UK. This includes the progenitor or equivalent TBOs from the same species. These comparators should display a similar trait to the altered trait in the PBO.

All compositional data, whether derived from a comparator or from a published dataset, must be relevant to the PBO trait and the species. When comparators from the same species are not available, a close relative to the species may be an acceptable comparator (for example, wheat, spelt, barley are related species in a same primary gene pool that can inform each other's compositional ranges).

5. Compositional considerations

Applicants must identify significant changes in the identity, quantity, and activity of intentionally targeted substance(s) (see Definitions), in addition to substances which could reasonably be anticipated to be altered as a result of the genetic change. These may arise directly from the genetic change, or result from linked traits. Applicants must determine the relevance of all significant compositional changes to the nutritional quality/safety of food/feed.

A compositional change is significant if it is outside the ranges found in traditionally bred comparators that have a HSFU in the UK or EU, or outside the ranges found in food composition dataset such as McCance and Widdowson (2021). PBOs with a significant compositional change include:

- Those which are known to, or likely to contain substances with no HSFU or in the UK or EU;
- PBOs containing quantities beyond the ranges found in equivalent comparator organisms such as biofortified PBOs;
- PBOs containing significant structural changes in allergens, toxins, nutrients or anti-nutrients altering activity such that there is no TBO equivalent with a HSFU or PFC in the UK or EU;
- A change in related pathways resulting in quantities of substances beyond the ranges found in equivalent comparator organisms such as biofortified PBOs; including changes in pathways related to bioaccumulation such as modifications to biological transporters.

All significant compositional changes must be assessed in the Tier 1 safety assessment, for which detailed guidance can be found in the relevant subsections for Nutrition (10), Toxicity (11), Allergenicity (12) and Other Safety Concerns (13).

Each PBO must be assessed on its own merits, and in the context of known hazards associated with the species. If an OECD consensus document (OECD, live database) is available for the crop, applicants may refer to the relevant consensus document for information on known toxicants, allergens, anti-nutrients and other plant metabolites. Compositional analysis may include modern techniques when appropriate, but regardless of the technique used, applicants are required to consider the potential consequences of any substantive change in composition on the food-safety management systems used by major anticipated processors (see Definitions).

5.1. Direct effects

Direct effects result from the proximate, molecular characteristics of the intended phenotype. From the description of change, applicants must identify direct changes to any allergens, toxins, nutrients and antinutrients.

Applicants must use their data in conjunction with their knowledge of the organism and the genetic change(s), alongside available peer reviewed scientific literature, to evaluate the relevance of the direct compositional changes.

If any significant direct compositional changes are identified, applicants must assess the changes by referring to the relevant subsections for Allergenicity, Toxicity Nutrition, and Other Safety Concerns.

5.2. Secondary effects

The genetic change may also affect composition indirectly by changing how the organism is grown, processed, or consumed. Applicants must evaluate the relevance of any reasonably anticipated secondary effects resulting from these by reference to the available peer reviewed scientific literature. Applicants may support their conclusions using data from any trials conducted to assess agronomic or technological function, if the methodologies and analyses used are suitable to provide an assessment of the compositional effects of agronomic or processing changes (see Section 5.3).

5.2.1. Cultivation and harvest

Applicants should only consider whether the change in cultivation or harvesting of the crop is likely to result in a significant compositional change affecting the consumed parts of an organism when the genetic change is intended to modify an agronomic function. Conclusions must be based on evidence and experience.

- Consider the impact of changes to harvest times, and/or stage of maturity on nutrient and antinutrient levels in Section 10 (Nutrition);

- Consider the impact of changes to the growing environment such as season, soil and climatic conditions, the presence of any contaminants, or environmental stress responses in Sections 11 (Toxicity) and 12 (Allergenicity).

Conclusions must be based on a sound scientific rational; when uncertain, evidence such as industry practice, international standards, economic data, scientific literature can be used.

5.2.2. Processing

Applicants are expected to consider whether the genetic change is intended or reasonably anticipated to alter the way in which a PBO is processed and if the change in processing is likely to adversely affect the consumer. For example, if a plant which must usually be cooked to be consumed was modified in a way that made it possible to eat raw, this would remove a Critical Control Point (cooking) used to reduce microbiological hazards. **Consider during the Tier 1 safety assessment** in Sections 10 (Nutrition), 11 (Toxicity) and 12 (Allergenicity).

Applicants must evaluate the implications that the intended phenotype in the PBO may have on the food safety management systems of intended post-harvest processors. Applicants should refer to food safety management systems used by major anticipated processors when evaluating Allergenic, Microbiological and Toxicological Hazards. **Consider during the Tier 1 safety assessment** in Sections 11 (Toxicity), 12 (Allergenicity) and 13 (Other Safety Concerns).

Consider whether any anticipated change to processing will affect digestibility of the feed product, and/or if nutrient bioavailability is changed. If digestibility or nutrient bioavailability is potentially affected, consider during Tier 1 safety assessment for Nutrition. If storage times, temperatures and light conditions are intentionally/significantly altered, applicants must consider how the changes influences nutrient and allergen content. **Consider during the Tier 1 safety assessment** in Sections 10 (Nutrition) and 12 (Allergenicity).

Where the PBO is intended to be used as a source for a food supplement, applicants must consider how remaining parts of the PBO may enter the food/feed chain during the Tier 1 safety assessment. Conditions of use may restrict entry into the food/feed chain to specific parts of the PBO. Food supplements put on the market must be compliant with regulations that apply (as listed on the FSA website on Food Supplements).

5.2.3. Consumption

If as a result of the genetic change, the way in which the organism is consumed changes (for example, raw instead of cooked), and/or the organism is consumed in different amounts, and/or the target population changes, there may be nutritional consequences for affected populations. **Applicants should consider during the Tier 1 safety assessment** in Section 10 (Nutrition).

5.3. Compositional data sources and sampling plan

Where the intention of the change is to alter the levels of substances impacting nutrition, toxicity, or allergenicity, analytic data must be obtained to substantiate the change. Further compositional data may also be needed in the Tier 2 safety assessment. The data used to support the compositional analyses and conclusions should reflect commercially-relevant growing conditions. All tests and analyses should be performed competently with suitable quality controls in place, in accordance with relevant standards such as ISO 17025 (2021). Testing facilities should be accredited by a competent authority such as the United Kingdom Accreditation Service (UKAS). Applicants should also adhere to OECD guidelines on Good Laboratory Practice, and Chemical Testing. Applicants may also consult the Summary of Key Considerations for chemical analysis (Institute of Food Science Technology, 2021) when designing their testing methodologies. Applicants should also consult any industry standards relevant to the substance of interest.

Applicants may use data collected during other studies providing there is a sufficient number of representative samples to determine the relevance of any compositional change.

Applicants are advised to retain samples where possible for additional analysis, should further compositional data be requested.

Applicants must ensure that samples are selected using an appropriate strategy. An appropriate sampling plan will have a sound scientific rationale, reflect real-world growing conditions and possess sufficient statistical power. Applicants should ensure that all experimental procedures are conducted according to Good Experimental Practice. Guidance on Good Experimental Practice can be found in sections 3.1-3.4 of the European and Mediterranean Plant Protection Organization (EPPO) Standard PP1/181 (2022).

The following key factors must be addressed:

- **Choice of comparator:** see Section 4.
- **Propagative material:** All propagative material used should be produced under similar environmental and storage conditions. Origin, year of production and production conditions should be as homogenous as possible for both the PBO seeds and the reference variety.
- **Propagative material health:** Propagative material should be of phytosanitary quality (see current phytosanitary requirements in the UK).
- **Test Material Suitability:** Testing material should be produced according to international standards (for example, International Seed Testing Association (2014) rules).
- **Site Selection:** Sites should be typical growing regions and conditions under which the PBO crop will be cultivated for food or feed use. For further

information, applicants may wish to consult EPPO guidance on comparable climates (EPPO, 2014).

- **Growing Seasons:** Should be representative of the different meteorological conditions under which the PBO crop will be cultivated for food or feed use.
- **Description of the receiving environments:** For aquaculture, provide additional details including, where relevant, the composition of the growing medium used, water use, any herbicides, and details of any additives such as perlite, vermiculate.
- **Endpoints:** Appropriate compositional and phenotypic endpoints must be used for comparative analyses. Particularly, phenotypic data that are linked to allergenicity, toxicity and nutrient quality.
- **Number of samples analysed.** Applicants must state the number of samples (e.g., plants) used for individual analysis. The number of samples must be large enough to provide sufficient statistical power. **A minimum of 5 representative samples independently harvested should be selected for analysis.**
- **Alterations in growth conditions**. Applicants must discuss any alteration in growing conditions from those typically used for the comparator. For example, less fertiliser, herbicide, or water use than would be used in the comparator species.

5.4. Reporting of compositional data

Where required, compositional data must be presented in the relevant section(s) (Nutrition, Toxicity, Allergenicity, Other Safety Concerns) in support of the analyses and conclusions made, and must include:

For a Regulation 20 application, the mean, range and standard deviation for the PBO and for its comparator, the number of representative samples used (a minimum of 5 representative samples independently harvested should be used), a description of the statistical methods used, and results of the statistical analysis. Analytical data for Tier 1 safety assessment should solely document the intended compositional change to demonstrate that the intended phenotype has been achieved.

For a Regulation 22 application, the raw data for a minimum of 5 representative samples of the PBO independently harvested and of its comparator, submitted in the form of a table; when mean is used for comparison, a description of the statistical methods used, and results of the statistical analysis. Supporting certificates of analysis should also be provided. Analytical data for Tier 2 safety assessment must document both the intended compositional change to demonstrate that the intended phenotype has been achieved, as well as any other substance as identified in Novelty, Nutrition, Toxicity or Allergenicity sections.

When analytical data from publications are used for comparative purposes, sufficient information must be available on the samples and methods utilised as well as on the laboratory where analyses have been carried out.

Under Regulation 33, the Secretary of State may consider revocation or variation of an authorisation, should new evidence come to light that calls into question the safety of the PBO as it is used in food and/or feed. In the event of any such evidence being made available, the authorisation holder will be given the opportunity to respond before an authorisation is revoked (Regulation 33 (4)). Therefore, it is recommended that authorisation holders retain any data used to support Tier 1 safety assessment should this be required to demonstrate safety at a future date.

6. Specific considerations

In addition to general and compositional considerations, there are specific considerations for PBOs that:

- Are novel (have no HSFU in the UK or EU prior to 15 May 1997)
- Are submitted as a batch application (see Section 6.2)
- Require new conditions of use to be applied that are not historically associated with the species and are not currently applied via other requirements in food/feed law
- Are intended for feed use or may enter the feed chain

When conducting a Tier 1 safety assessment, applicants must ensure any possible concerns related to the following relevant specific considerations are addressed throughout the application.

6.1. Novelty

History of safe food use (HSFU) is determinant of Novelty. HSFU means that "the safety of the species in question as food has been confirmed with compositional data and from experience of continued food use in the customary diet of a significant number of people in the United Kingdom or the European Union beginning before 15th May 1997" (Regulation 20 (2)). When the progenitor organism of a PBO for food does not have a HSFU, the PBO requires a Tier 2 safety assessment for Novelty (see Section 25). This will require a high-level data submission on the PBO consistent with existing Novel Food regulations. There are two approaches to safety assessment dependent on whether the PBO is determined to be novel according to the Novelty criterion:

- When an applicant cannot demonstrate that the PBO belongs to a species with a HSFU, the PBO is considered to be Novel for food use, and therefore requires an FSA safety assessment; applicants must also complete the Tier 1 safety assessment for Other Safety Concerns;
- When an applicant can demonstrate that the PBO belongs to a species with a HSFU, the PBO is considered to be not Novel for food use, and Tier 1 safety

assessment for Nutrition, Toxicity, Allergenicity and Other Safety Concerns need to be completed by the applicant.

Production processes not used for food production within the UK or EU before 15 May 1997, and which give rise to significant changes in the composition or structure of a food, need in-depth safety assessments. Where such a novel production process is intended to be used in conjunction with the genetic change to produce a food, this does not require a Tier 2 safety assessment under Novelty; instead it will require a Tier 2 safety assessment under Other Safety Concerns (see Section 13.2.2) using information similar to what is required in section 2 of the EFSA Guidance for Novel Foods (2024c).

When a PBO is used as source for a substance that was exclusively used as a food supplement in the UK or EU before 15 May 1997, this does not require a Tier 2 safety assessment under Novelty; instead, applicants must follow the Tier 1 safety assessment described in Sections 10.2 (Nutrition), 11.2 (Toxicity) and 12.2 (Allergenicity).

Feed uses do not require a Tier 2 safety assessment under Novelty; instead, they must always be safety assessed as part of Tier 1 safety assessment for Nutrition, Toxicity, Allergenicity and Other Safety Concerns as described in Sections 10, 11, 12 and 13.

6.2. Batch applications

A single precision bred Defra Marketing Notice (see Definitions) can serve as a notice for more than one PBO provided they belong to the same species as the initial PBO and meet the criteria in Regulation 5 (4).

Batch food and feed marketing authorisation applications may be sought for the PBOs included in a same Defra Marketing Notice. Batch applications must detail the differences in genetic changes in food safety considerations between the individual varieties within the batch, in accordance with the requirements set out in Schedule 4 (1) (3) (d) and (1) (4).

6.3. Conditions of use

If, as a result of the genetic change, the organism requires new conditions of use be applied in addition to any existing, historical condition(s) of use for organisms of the same species, these must be considered. Applicants must provide any relevant information to support the FSA safety assessment and consideration of risk management options of the new variety (see Section 13.2.1). This will require a Tier 2 safety assessment under Other Safety Concerns.

All parts of the plant historically known to enter food or feed chain must be taken into consideration in the safety assessment of the PBO, unless conditions of use restrict the use to specific parts of the organism.

If an application is made for feed use only, applicants must provide any relevant information to support the determination of appropriate conditions of use under Regulation 30 to prevent the entry of the PBO into the human food chain.

6.4. Feed

Where PBOs are expected to be consumed by livestock, specific feed uses should be considered during Tier 1 safety assessment.

Animal feed may be produced from a single organism which may therefore constitute a significant portion of an animal's diet. For instance, 50 to 75 percent of the diet of most livestock animals can consist of a single plant species. Compositional changes to feed can therefore have a greater impact on the overall diet of the animal, which in turn affects both animal condition and the nutritional quality of food products produced by, or derived from the animal. Applicants must be aware of other regulations on feed (as listed on the FSA webpage) that apply.

Similarly, attention must be given to changes in digestibility. Poor digestibility may negatively impact nutrient bioavailability in the target livestock. This is particularly relevant where the feed consists of parts of an organism which humans do not consume.

Consideration should be given to any intended or reasonably anticipated changes to feed preparation which may adversely affect the feed nutritional quality. While a PBO may be designed with food use in mind, by-products of crops are often repurposed for feed.

7. Part 1 Concluding remarks

Part 1 outlined the purpose and scope of this Guidance and introduced the basic principles of the tiered safety assessment for PBOs leading to either Regulation 20 or Regulation 22 applications.

The Tier 1 safety assessment described in Part 2 (Applicant-led Tier 1 safety assessment) focuses on the need to understand and explain compositional data and expected use, and to provide assurance that considerations of safety of food and feed have been addressed by applicants. Part 2 describes each step of this process with flow charts to determine whether a PBO requires a Regulation 20 application to the FSA or whether it requires a more detailed Regulation 22 application.

The following sections provide detailed guidance regarding what information needs to be included in all applications (Part 3) and what additional information must be included in Regulation 22 applications (Part 4).

Applicants are responsible for the decisions taken and the information provided in this process. Where there are uncertainties regarding any of the criteria set out in Regulation 20 (1) impeding accurate assessment of food and/or feed safety, a Regulation 22 application must be made. An application incorrectly submitted under Regulation 20 where further assessment is necessary to demonstrate safety may face significant delays and/or rejection. The existing statutory obligations require food and feed businesses to ensure the food and feed they place on the market is safe. The FSA will verify whether Regulation 20 applications contains all the required information and will take action where it considers that applicants have not exercised the adequate level of due diligence in considering the safety of their PBO in line with this guidance. In some cases, the FSA will seek further intormation from applicants in accordance with Regulation 24 as part of the verification process.

Part 2 – Applicant-led Tier 1 safety assessment

8. Introduction to Tier 1 safety assessment

Applicants must answer each of the five safety assessment questions in the Tier 1 safety assessment, except when Tier 2 safety assessment is required for Novelty (see Section 6.1). The Tier 1 safety assessment focuses on changes to composition in regard to the following criteria:

- **Novelty** – Food which contains or consists of, or is otherwise derived from PBOs will remain outside of the scope of the existing regulatory regimes for novel foods. However, it is possible that a PBO could be generated by precision breeding of a progenitor that has not been consumed to a significant degree in the UK or EU prior to 15 May 1997. In these cases, further assessment with a similar degree of safety assessment to the approach of the novel food regulatory regime is required. This ensures consumer safety and legislative consistency.
- **Composition (nutrition, toxicity, or allergenicity)** – Understanding the phenotypic consequences of the genetic change(s) in a PBO is essential in determining its safety. Knowledge of the resultant phenotypes allows assessment of changes that may be nutritionally disadvantageous for the consumer, and of potential significant changes to the toxicity or allergenicity of food or feed made from the organism. The Tier 1 safety assessment focuses on intended effects, but reasonably anticipated changes (see Definitions) must also be considered.
- **Other safety concerns** – A wide range of traits can be altered or introduced into a PBO. PBOs with changes that may impact safety in ways not covered by compositional assessment, or that enable uses that may cause an identifiable food safety issue, must be considered in Other Safety Concerns.

Each section of the guidance must be navigated by completing the sub questions, where “yes” or “no” answers will decide whether a Tier 2 safety assessment is required for the corresponding criteria. Conclusions drawn during Tier 1 safety assessment are then provided in the data submission (Figure 2).

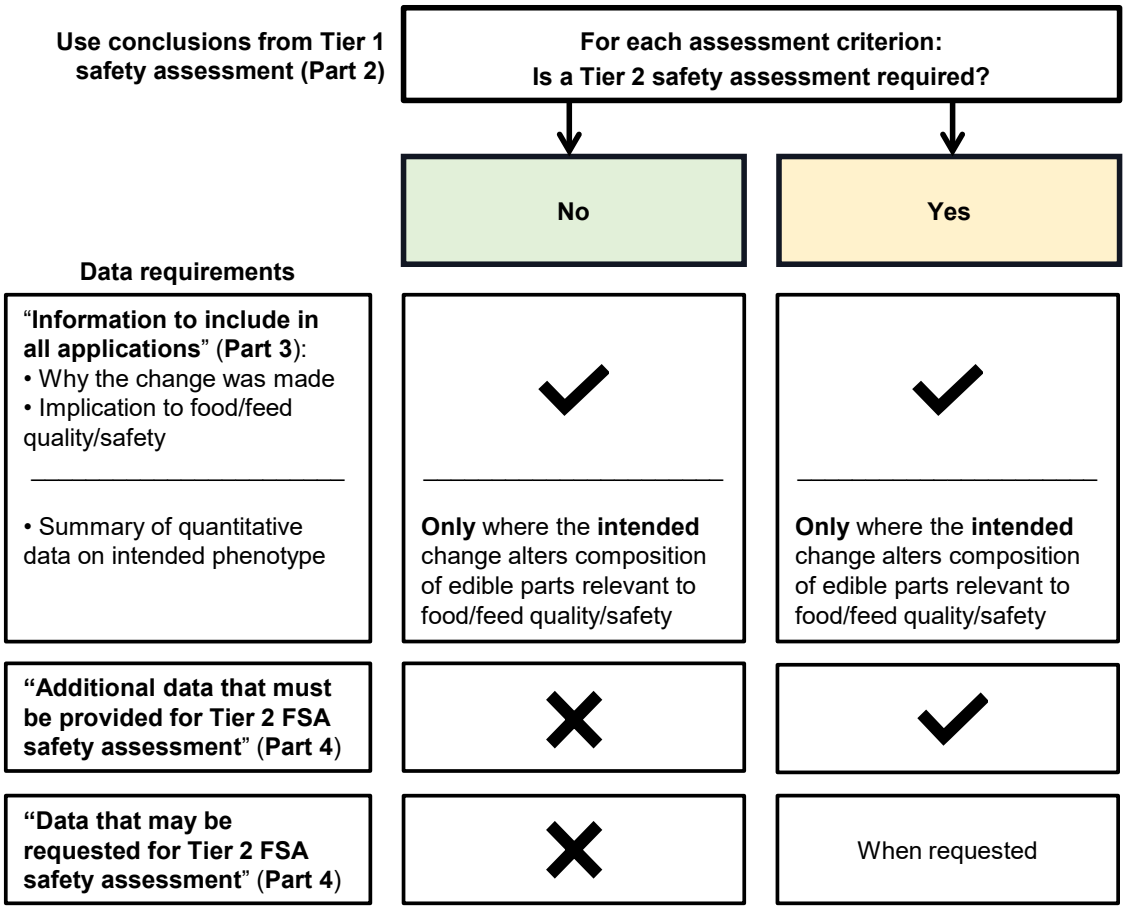


Figure 2.
Flowchart outlining the details of the tiered safety assessment process which apply to each assessment criterion. For each criterion, applicants complete the Tier 1 safety assessment described in Part 2 of the guidance and determine whether a Tier 2 safety assessment is required. Applicants must then submit the appropriate level of data for each criterion to support the required level of safety assessment.

9. Novelty Tier 1 safety assessment

9.1. Introduction to Novelty

This part of the guidance specifically addresses the requirement in Regulation 20 (1) (b): "the applicant is able to demonstrate that the relevant precision bred organism belongs to a species that has a history of safe food use."

A "history of safe food use" (HSFU) is defined in Regulation 20 (2) as where "the safety of the species in question as food has been confirmed with compositional data and from experience of continued food use in the customary diet of a significant number of people in the United Kingdom or the European Union beginning before 15th May 1997."

The Novelty Tier 1 safety assessment requires answering the safety assessment question: "**Is the PBO from a species that has no history of safe food use in the UK or EU?**" as described in Figure 3.

TBOs for food use that have no HSFU are subject to Novel Food assimilated Regulation (EU) 2015/2283. However, following the implementation of the Genetic Technology (Precision Breeding) Act, a consequential amendment to the Novel Food assimilated Regulation keeps Foods which contain or consist of, or are otherwise derived from PBO plants out of scope from the Novel Food regulations.

Under Novel Food assimilated Regulation (EU) 2015/2283, where a food which would otherwise be a novel food is a "traditional food from a third country", a notification procedure may in some circumstances allow the food to be authorised without a safety assessment. However, FSA's experience has shown that a safety assessment or additional review is required in most cases of traditional foods from third countries being used in the UK diet. Therefore, all PBOs for food from organisms without a HSFU in the UK or EU require a Tier 2 FSA safety assessment as described in Section 25, however the type and amount of information to consider for the FSA-led safety assessment will depend on whether the PBO is from an organism with traditional use for food in a third country (PB-OTU) or from a novel organism for food use (PB-NvO).

For traits that are new to the PBO, a closely related species with the same trait and with a similar role in the diet, that has a HSFU, can inform conclusions on the safety of the trait, and whether Tier 2 safety assessment is needed for the compositional and "Other Safety Concerns" criteria (Sections 10.2, Steps (**1**) and (**6**); 11.2, Step (**7**); 12.2, Step (**5**); 13.2.3). In such cases, any HSFU must relate to the same form of use of the PBO, for example whether the same parts of the organisms are to be used, or whether the role in the diet will be equivalent.

History of safe food use for the trait introduced by PB may be provided when, for example:

- Homologous genes exist in closely related species (where the function of an introduced cisgene is novel to the host species);
- Food and/or feed products or organisms containing an equivalent trait or mutation in homologous gene(s), and with the same function in the diet, are already on the market.

Feed produced from PBOs where the progenitor organism has never been used to produce **feed** before must be compliant with the requirements laid down in feed legislation (see Section 6.4). When PBOs are developed for feed use or may be used for feed, applicants should adhere to the statutory duties to ensure that the feed they produce and place on the market is safe. PBOs from species with no prior feed consumption (PFC) must undergo a Tier 1 safety assessment for the compositional and "Other Safety Concerns" criteria as described in Sections 10, 11, 12 and 13.

PBOs intended for food use only, for feed use only, or for both food and feed use, require different approaches to the tiered assessment.

9.2. How to perform a Tier 1 safety assessment for Novelty

Where a PBO is intended for food use, part A of the safety assessment must be completed (Section 9.2.1); where a PBO is intended for feed use, part B of the safety assessment must be completed (Section 9.2.2); where a PBO is intended for both food and feed use, parts A and B of the safety assessment must be completed.

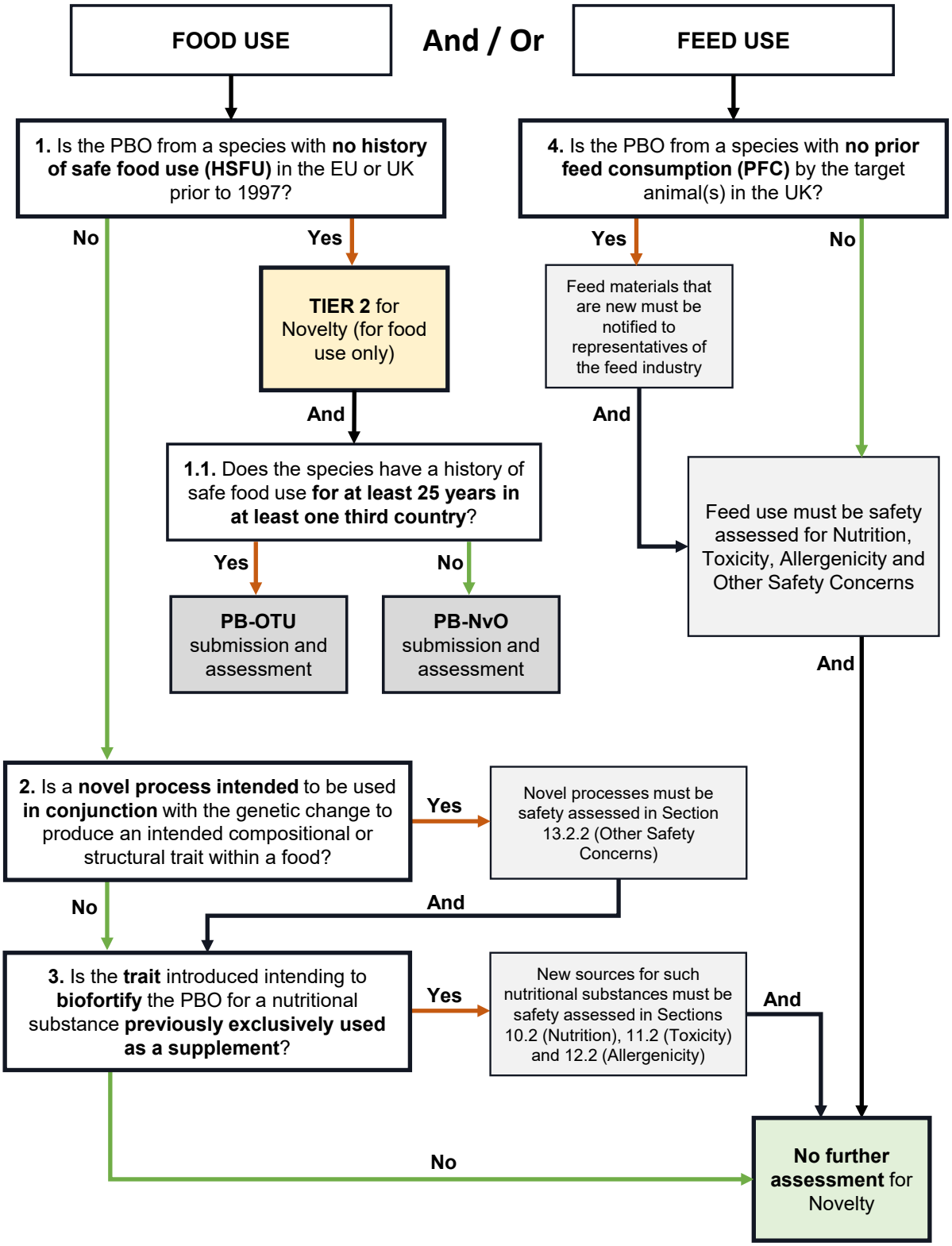


Figure 3.

Flowchart outlining the Tier 1 safety assessment process used to answer the safety assessment question about Novelty: "**Is the PBO from a species that has no history of safe food use in the UK or EU?**" There are two paths to consider depending on if the PBO is intended for use as food or feed; both paths must be followed if both uses are seeking authorisation. (**i**) **For food use**, the Information and Guidance document on "human consumption to a significant degree" (Council of the European Union, 2018) can assist in determining whether there is a HSFU. When

a PBO for food use does not require a Tier 2 safety assessment for Novelty, applicants must also complete the Tier 1 safety assessment for Nutrition, Toxicity, Allergenicity and Other Safety Concerns. When the novelty for food use of a PBO requires a Tier 2 safety assessment, applicants only need to also complete the Tier 1 safety assessment for the 'Other Safety Concerns' criterion; this is because compositional assessment for PBOs from Organisms with traditional use in food (PBs-OTU) or for PBOs from novel organism for food (PBs-NvO) is completed as part of the FSA-led safety assessment. For detailed instructions, refer to Section 9.2.1. (**ii**) **Feed use** does not have a Tier 2 safety assessment for Novelty. When a PBO is for feed use, applicants must complete the Tier 1 safety assessment for Nutrition, Toxicity, Allergenicity and Other Safety Concerns. Where the Tier 1 safety assessment concludes that a Tier 2 safety assessment is required, a Regulation 22 application must be made. For detailed instructions, refer to Section 9.2.2.

9.2.1. Part A – Tier 1 safety assessment of novelty for PBOs for food use

Step (1) – Is the PBO from a species with no history of safe food use (HSFU) in the EU or UK prior to 1997?

The safety of an organism for food use is supported by compositional data and from the experience of continued food use in the customary diet of a significant number of people **in the EU or UK** before 15 May 1997 (HSFU). For the purpose of its assessment of novelty, the FSA takes into account the guidance from the Food Safety European Commission (Information and Guidance document on "human consumption to a significant degree" (Council of the European Union, 2018), to determine where consumption is sufficiently significant to establish a HSFU.

If the answer is Yes: A Tier 2 safety assessment for Novelty is required and a Regulation 22 application must be made; this ends the safety assessment of novelty of the food use of the PBO. Proceed to Step (**1.1**). Also complete Tier 1 safety assessment in Section 13 (Other Safety Concerns), but not in Sections 10 (Nutrition), 11 (Toxicity) and 12 (Allergenicity).

If the answer is No: Where the PBO is of a species with a HSFU in the EU or UK, the PBO does not require Tier 2 safety assessment for Novelty as described in Section 25, but aspects that may introduce new and additional risks also need to be considered: proceed to Step (**2**).

- **Step (1.1) – Does the species have a history of safe food use for at least 25 years in at least one third country?**

  Experience of continued food use in a third country for at least 25 years from the date of application may indicate a history of safe food use and support the safety of a species as a source of food. This may mean the safety assessment can be less detailed or in-depth in certain areas. In contrast, newly domesticated species would not benefit from any history of use prior to 1997.

If the answer is Yes: An FSA safety assessment of the PB-OTU, similar to Traditional Foods from third countries in the context of assimilated Regulation (EU) 2015/2283, but taking into account the phenotype resulting from PB, is required; the information to be provided is described in Section 25. However, applicants seeking an authorisation of a PBO-OTU not limited to its traditional food uses should provide the information required for a PBO-NvO. Where the PBO intended use also **includes feed**, also complete Step (**4**).

If the answer is No: An FSA safety assessment of the PB-NvO, similar to other Novel Foods in the context assimilated Regulation (EU) 2015/2283; this requires the providing of information described in Section 25. Where the PBO intended use also **includes feed**, also complete Step (**4**).

Changes which are likely to require a non-traditional type Tier 2 FSA safety assessment for Novelty include those made in the context of *de novo* domestication of a wild species not commonly consumed:

- There would be uncertainty about composition (including the possible presence of substances not known to be normally present in the diet) and the nature of any potential safety concerns arising in the host organism.
- Multiple genome edits to a wild species to obtain the desirable domesticated traits (for example, improvement of crop yield, making the organism or its products more edible/attractive), leading to significant (and multiple) phenotypic differences between the PBO and the wild progenitor, may further increase uncertainty about composition and potentially impact risk.
- *De novo* domesticated species could change their adaptation to a certain climate/environment leading to, for example, altered levels of toxic substances, justifying further safety assessment.

Step (2) – Is a novel process intended to be used in conjunction with the genetic change to produce an intended compositional or structural trait within a food?

A production process is novel when it gives rise to significant changes in the composition or structure of a food, affecting its nutritional value, metabolism or level of undesirable substances, **and** it has not been used for food production **within the UK or EU** before 15 May 1997 (Article 3 (2) (a) (vii), assimilated Regulation (EU) 2015/2283).

Some PBO may require the use of a specific processing step to fully achieve the intended phenotype (for example, UV treatment – see Section 13.2.2); other traits may be introduced specifically to allow the PBO or a part of it to be processed using a new technique (for example, extraction technique – see Section 13.2.2). Where a novel process is needed, this requires a Tier 2 FSA safety assessment under Other Safety Concerns (Section 13.2.2).

If the answer is Yes: Where the PBO is intended to be made into food using a production process that is novel introducing significant changes to the composition or structure of the food made of it, Tier 1 safety assessment as described in Section 13.2.2

(Other Safety Concerns) of this technical guidance must be followed. Also proceed to Step (**3**).

If the answer is No: Proceed to Step (**3**).

Step (3) – Is the trait introduced intending to biofortify the PBO with a substance previously exclusively used as a supplement?

In accordance with the Food Supplements (England) Regulations (2003), "food supplements" means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form.

For foods from TBOs: under assimilated Regulation (EU) 2015/2283, any food (which includes vitamins, minerals and other substances) used exclusively in food supplements within the UK or EU before 15 May 1997, where it is intended to be used in foods other than supplements (as defined in point (a) of Article 2 of Directive 2002/46/EC), is a novel food (Article 3 (2) (a) (x)) and would need to be assessed under that regime.

For foods from PBOs: where the intention of the genetic change(s) is to allow production in the PB plant of a substance which was not used in foods other than food supplements within the UK or EU before 15 May 1997, the PBO which has become a new dietary source for this substance is submitted to a tailored nutritional and toxicity Tier 1 safety assessment by the applicant to determine whether a Tier 2 FSA safety assessment is needed.

If the answer is Yes: Where the PBO is intended to be used as a new dietary source for a substance previously provided in the form of supplements, this must be taken into consideration in the Tier 1 safety assessment in Sections 10.2 (Nutrition), 11.2 (Toxicity) and 12.2 (Allergenicity). This ends the Tier 1 safety assessment of novelty for food use, no further safety assessment is required for Novelty. Where the PBO intended use **includes feed**, also complete Step (**4**). Where the PBO intended use does not include feed, proceed to Section 10.

If the answer is No: This ends the Tier 1 safety assessment of novelty for food use, no further safety assessment is required for Novelty. Where the PBO intended use **includes feed**, also complete Step (**4**). Where the PBO intended use does not include feed, proceed to Section 10.

9.2.2. Part B – Tier 1 safety assessment of novelty for PBOs for feed use

Step (4) – Is the PBO from a species with no prior feed consumption (PFC) by the target animal(s) in the UK?

While a PBO may be designed with food use in mind, by-products of crops are often repurposed for feed. The use of novel organisms for food is therefore likely to result in the use of feed material with no or little prior consumption by animals.

Because feeds that are from species new for use in feed are not subject to Novel Food regulations for non-PB organisms, they are not required to undergo an FSA safety assessment for Novelty; instead, feed businesses are expected to exercise due diligence in considering the safety risks feed products may present. When PBOs are developed for feed use or may be used for feed, and are from species with no PFC, they must be further safety assessed through the compositional and 'Other Safety Concerns' sections of this guidance. The outcome of Tier 1 safety assessment of novelty for PBOs **for feed use** will never be a requirement of Tier 2 safety assessment for Novelty, and the correct regulatory route will be determined by the responses to the other assessment criteria.

Assimilated Regulation (EC) No 767/2009 on the placing on the market and use of feed requires that new feed materials must be notified to representatives of the feed industry and registered on the GB Register of Feed Materials. It is the responsibility of the person who places the feed material on the market for the first time to complete this notification immediately.

If the answer is Yes: Where the PBO is of a species with no PFC by target animals, the new feed material must be notified to representatives of the feed industry. Also complete Tier 1 safety assessment as described in Sections 10 (Nutrition), 11 (Toxicity), 12 (Allergenicity) and 13 (Other Safety Concerns) of this technical guidance. Proceed to Section 10.

If the answer is No: Where the PBO is of a species with significant PFC by target animals in the UK or EU, proceed to Section 10.

10. Nutrition Tier 1 safety assessment

10.1. Introduction to Nutrition

This part of the guidance specifically addresses the requirement in Regulation 20 (1) (c) (i): "The applicant is able to demonstrate that the application of modern biotechnology does not introduce genetic changes that are expected to significantly alter the nutritional quality of the organism currently consumed that are likely to be disadvantageous to the consumer."

Changes in nutritional quality cannot be examined in isolation. Nutritional quality is a combination of multiple interrelated factors, including nutrient content digestibility, bioavailability and the contribution of the PBO to the diet. Any nutritional change will be affected by intended use, processing, storage, HSFU/PFC and nutrient bioavailability. There are numerous modifying factors that may exacerbate or ameliorate any potential risks associated with targeted and anticipated changes.

Applicants must review how the introduced trait could impact the nutritional quality of food and/or feed. Applicants must provide the conclusion of their review including descriptions of all supporting scientific evaluations of how nutritional quality and safety profile may be significantly altered.

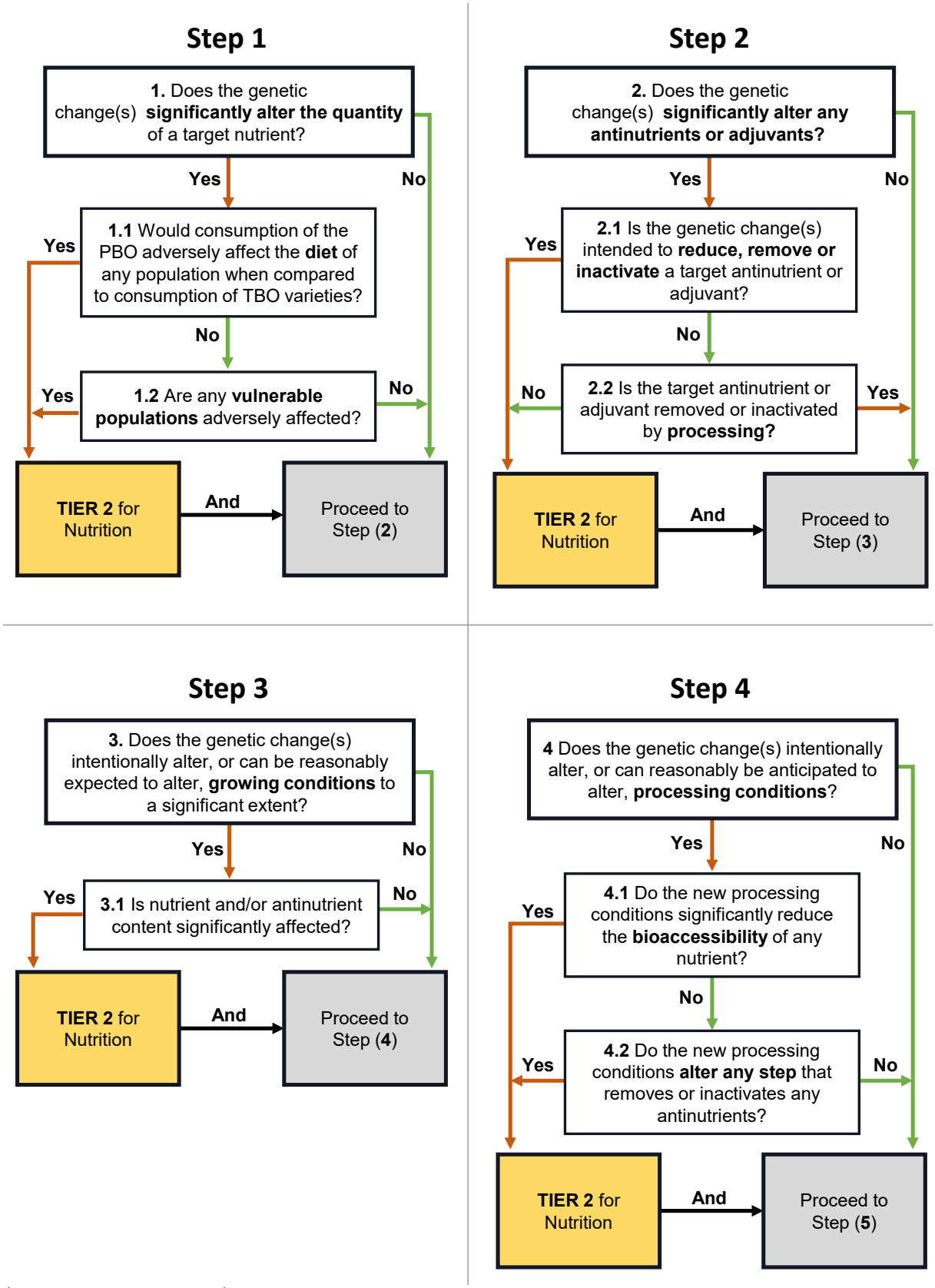
Where authorisation is sought for multiple PBOs as part of a batch (see Section 6.2), each question must be considered for all PBOs within the batch. Any difference in nutrition expected between the different PBOs within the batch must be clearly identified for each question.

10.2. How to perform Tier 1 safety assessment for Nutrition

The Nutrition Tier 1 safety assessment requires answering the question: "**Is the PBO designed to introduce significant changes to the nutritional quality of the organism currently consumed that are likely to be disadvantageous to the consumer?**" as described in Figure 4. This means addressing intended, reasonably anticipated and secondary effects. Answering this question about Nutrition involves identifying the changes in nutritional quality and understanding their impact by comparison to a suitable comparator. Different comparators may be selected for different purposes. A suitable comparator for processing may include a TBO variety of the same species, that has a HSFU/PFC, and shares the same processing properties as the PBO (see Section 4).

This section guides applicants through the steps outlined in Figure 4. Each step should be answered in sequence. Step (**1**) focuses on intentional nutritional changes, Steps (**2**)-(**5**) focus on secondary effects on nutrition, and Step (**6**) focuses on reasonably anticipated changes to nutrition. Where any of their responses to questions outlined in the flowchart require a Tier 2 FSA safety assessment, applicants must still complete

the Tier 1 safety assessment process described in the rest of the flowchart. It is possible that more than one response may require a Tier 2 FSA safety assessment.



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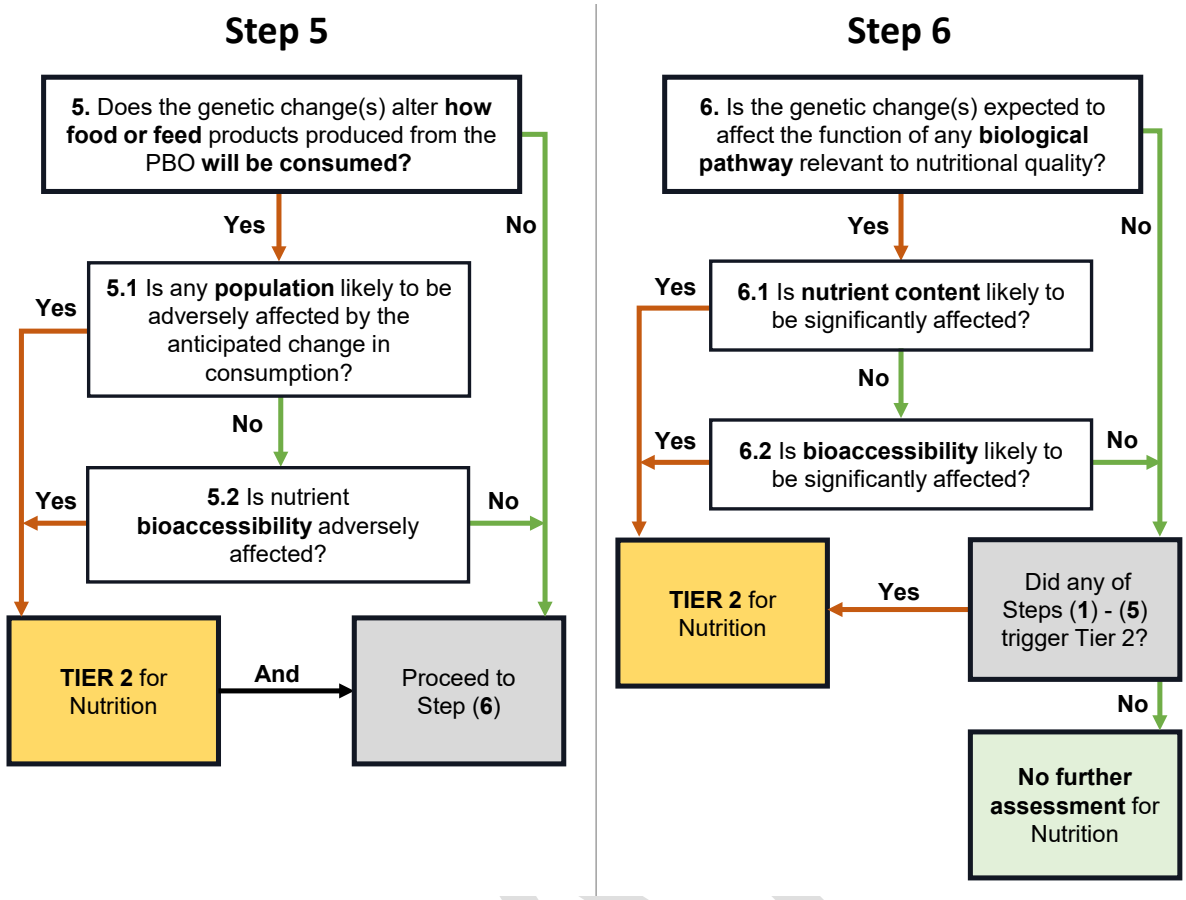


Figure 4.

Flowchart outlining the Tier 1 safety assessment process used to answer the safety assessment question about Nutrition: "**Is the PBO designed to introduce significant changes to the nutritional quality of the organism currently consumed that are likely to be disadvantageous to the consumer?"** A nutritional change is significant if it is above existing Safe Upper Limits (SUL), or outside the ranges found in reference food composition datasets, or outside the ranges found in suitable comparators that have a HSFU/PFC in the UK or EU, and is biologically relevant to safety. Where the Tier 1 safety assessment concludes that a Tier 2 safety assessment is required, a Regulation 22 application must be made. For detailed instructions, refer to Section 10.2.

Step (1) – Does the genetic change(s) significantly alter the quantity of a target nutrient?

Significant alterations in quantity include those resulting from the introduction of a nutrient that is new to the organism (for example as a result of the introduction of new genes from closely related species by cisgenesis or intragenesis), or of a nutritional substance previously provided to the diet in supplements only (see Section 9.2 Step (**3**)). Where any substance(s) produced are new to the organism, they must be assessed for effects on the diet. This is due to the absence of any HSFU or PFC of the PBO as a dietary source of these substances.

If the answer is No: Proceed to Step (**2**).

If the answer is Yes: Identify which nutrient has been altered or introduced and provide compositional data quantifying the target nutrient and related substances. Applicants must compare the nutritional content of the PBO to a suitable comparator to determine whether the PBO is nutritionally disadvantageous. A PBO may be considered nutritionally disadvantageous if the quantity of a nutrient is significantly reduced or increased beyond the range expected in TBOs such that typical consumption may cause harm. If the target nutrient is significantly increased, identify any potential health concerns associated with high levels of consumption by reference to the available peer reviewed literature. This information must also be considered in Step (**4**) of the safety assessment for Toxicity (Section 11.2.2). Estimates of daily intakes of the nutrient in relation to the Dietary Reference Values Upper Level must be undertaken together with consideration of any potential adverse effects on the bioavailability of other nutrients. Similarly, if nutrient levels are decreased, applicants must determine whether any vulnerable populations may be adversely affected as a consequence. Applicants must refer to relevant data sources such as the Expert Group on Vitamins and Minerals Report into Safe Upper Limits for Vitamins and Minerals (2003), the EFSA Guidance on Tolerable Upper Limits (2022) and the EFSA Dietary Reference Online Tool (2019). Proceed to Step (**1.1**).

For Example:

Biofortification. An applicant wishes to submit an application for a vitamin-enriched food crop. The applicant must quantify the change in the vitamin content using data reflecting industrially relevant conditions, provide details on the level of intake at which adverse health effects occur, and identify any potential adverse health effects from high levels of the vitamin.

Step (1.1) – Would consumption of the PBO adversely affect the diet of any population when compared to consumption of TBO comparators?

In addition to the initial analysis conducted in Step (1), applicants must determine whether any population would be nutritionally disadvantaged by consumption of the PBO. Applicants should compare the values in the PBO to typical reference values for the host organism and appropriate consumption databases such as the NDNS dataset (Public Health England, 2020), and the EFSA Comprehensive Food Consumption Database (EFSA, 2018).

When the target nutrient is a vitamin or mineral, a change in content would not be considered nutritionally disadvantageous if a single portion of the edible parts of the PBO and the comparator contain less than 15% of the nutrient reference value for the affected vitamin or mineral (Part A. 2. Annex XIII of assimilated Regulation (EU) 1169/2011.

For feed, applicants should be aware that any new feed must be entered onto the National Feed Registry, AIC | GB Register of Feed Materials, agindustries.org.uk, according to assimilated Regulation (EC) No 767/2009. New entries should provide a description of the key characteristics of the feed including details of the main nutrients.

If the answer is No: Proceed to Step (**1.2**).

If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. In their Regulation 22 submission, applicants must identify any affected populations and the role the PBO will play in the diet. Proceed to Step (**1.2**).

- **Step (1.2) – Are any vulnerable populations adversely affected?**

A vulnerable population is a group of people who are at greater risk of undernutrition than the general population. This includes infants, the elderly, pregnant and lactating women, and people suffering from illness. Vulnerable populations could be particularly affected if the PBO forms a key part of their diet. To answer the question, applicants must identify whether the PBO forms a key part of the diet of any vulnerable population by reference to appropriate consumption statistics such as the NDNS dataset (Public Health England, 2020). Applicants may also wish to consult relevant SACN reports and position papers

If the answer is No: Proceed to Step (**2**).

If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. Proceed to Step (**2**).

For Example: Provitamin A can in excess and in deficit cause an array of developmental abnormalities in the developing foetus. Therefore, a PBO with significantly altered vitamin A content when compared to a suitable comparator would need to a Tier 2 FSA safety assessment. Applicants must consider nutritional guidelines, and determine whether restrictions regarding consumption of the PBO during pregnancy is required if not already in place. Further information about vitamin and mineral exposure can be found in published NHS guidelines. Vitamins and minerals - NHS (www.nhs.uk)

Step (2) – Does the genetic change(s) alter any antinutrients or adjuvants?

Any alteration to antinutrients such as lectins, and adjuvants such as saponins or squalene must be evaluated for any adverse effects. Applicants must clearly state whether the abundance and/or potency of the antinutrient or adjuvant in the pre-processed PBO will be increased or reduced. Applicants must evaluate the effect of the genetic change on wider biochemical processes impacting antinutrient or adjuvant production, in addition to the intended and proximate effects. If the species has a known antinutrient hazard, applicants must evaluate whether the genetic change either intentionally significantly alters, or is reasonably anticipated to significantly alter the antinutrient content of the PBO.

If the answer is No: Proceed to Step (**3**).

If the answer is Yes: Proceed to Step (**2.1**).

- **Step (2.1) – Is the genetic change(s) intended to reduce, remove or inactivate a target antinutrient or adjuvant?**

  If the answer is No: Proceed to Step (**2.2**).

  If the answer is Yes: Applicants must produce data confirming that the antinutrient or adjuvant content has been significantly reduced compared to traditional varieties and reference lines, and/or inactivated. Proceed to Step (**3**).

- **Step (2.2) – Is the target antinutrient or adjuvant removed or inactivated by processing?**

  If the answer is No: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. Proceed to Step (**3**).

  If the answer is Yes: Identify the processing step(s) that remove or inactivate the antinutritional factor. Evaluate the efficacy of antinutrient removal and/or inhibition using appropriate supporting evidence (references, test results etc.). If all antinutrients are effectively removed or inactivated, proceed to Step (**3**).

Step (3) – Does the genetic change(s) intentionally alter, or can be reasonably expected to alter, growing conditions to a significant extent?

If the answer is No: Proceed to Step (**4**).

If the answer is Yes: Applicants must further evaluate the significance of the change in growing conditions to nutritional quality as outlined in Step (**3.1**). Changes to growing conditions are significant if the bioaccessibility (see Definitions) of a nutrient between crop and consumer is altered in a way that is nutritionally disadvantageous to the consumer. Proceed to Step (**3.1**).

- **Step (3.1) – Is nutrient and/or antinutrient content significantly affected?**

  Nutrient content is significantly affected if the quantity of a nutrient is reduced or increased, and the increase or reduction is likely to nutritionally disadvantage the consumer. Applicants must refer to an appropriate dataset such as McCance & Widdowson's (2021) to evaluate changes in nutritional content. If using commercially sensitive datasets, applicants must provide them to the FSA when required to demonstrate safety, but applicants can request for these to be treated as commercially confidential (Regulation 34). Applicants must consider possible impacts of altered growing conditions on nutrient content. Nutrient content may be affected by climate, changes to soil conditions, growing seasons, fertiliser use and time to harvest.

For Example: Crops that have decreased time to harvest may have a different nutritional profile due to reduced time assimilating nutrients. Applicants would then need to evaluate whether such a change is likely to be significant when compared to similar products.

If the species has a known antinutrient hazard, applicants must evaluate whether the new growing conditions are likely to significantly alter the antinutrient content of the PBO when compared to typical cultivation. Applicants should identify any new or increased biotic or abiotic stresses in the new growing conditions which may induce a changed stress response, thereby changing the antinutrient profile of the PBO.

If the answer is No: Proceed to Step (**4**).

If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. Proceed to Step (**4**).

Step (4) – Does the genetic change(s) intentionally alter, or can be reasonably anticipated to alter processing conditions?

If the answer is No: Proceed to Step (**5**).

If the answer is Yes: Applicants must identify the processing step(s) that have changed and evaluate the nutritional significance of each change using Steps (**4.1**) and (**4.2**) as a guide. Changes to processing conditions are nutritionally significant if the content or bioaccessibility of a nutrient is likely to be altered in a way that is nutritionally disadvantageous to the consumer. Proceed to Step (**4.1**).

- **Step (4.1) – Do the new processing conditions significantly reduce the bioaccessibility of any nutrient?**

  With reference to relevant peer reviewed research, assess the impact of the new processing conditions on nutrient content, bioaccessibility, digestibility and absorption.

  If the answer is No: Proceed to Step (**4.2**).

  If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. A Tier 2 FSA safety assessment will be conducted for the purposes of determining the safety impact of the new processing conditions. Proceed to Step (**5**).

- **Step (4.2) – Do the new processing conditions alter any step that removes or inactivates any antinutrients?**

  If the answer is No: Proceed to Step (**5**).

  If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. A Tier 2 safety assessment will

be conducted for the purposes of determining the safety impact of the processing change on antinutrient content and activity. Proceed to Step (**5**).

Step (5) – Does the genetic change(s) alter how food or feed products produced from the PBO will be consumed?

This must be identified in Section 16.2.4.

If the answer is No: Proceed to Step (**6**).

If the answer is Yes: If applicants have identified a change in how the food and feed products are consumed, including: the target population, the role of the food/feed products in the overall diet, and intended intake levels, applicants must evaluate the significance of the change using Steps (**5.1**) and (**5.2**) as a guide. Proceed to Step (**5.1**).

- **Step (5.1) – Is any population likely to be adversely affected by the anticipated change in consumption?**

  Estimate the anticipated change in consumption by reference to appropriate consumption databases such as the NDNS survey (Public Health England, 2020) and the EFSA Comprehensive Food Consumption Database (2018).

  Identify any potential health concerns associated with high levels of consumption by reference to the available scientific literature and review against upper tolerable limits and dietary recommendations. Applicants must determine whether their estimate of the anticipated change in consumption indicates a likelihood of adverse health effects (see Definitions). Particular attention must be given to how vulnerable populations are affected.

  If the answer is No: Proceed to Step (**5.2**).

  If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. Proceed to Step (**6**).

- **Step (5.2) – Is nutrient bioaccessibility adversely affected?**

  To assess bioaccessibility, applicants must consider factors affecting absorption including digestibility and antinutrient content.

  If the answer is No: Proceed to Step (**6**).

  If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. Proceed to Step (**6**).

Step (6) – Is the genetic change(s) expected to affect the function of any biological pathway relevant to nutritional quality?

A decision on this may be supported from the information required in Section 16.3 (Description of the genetic change(s)). Applicants must have sufficient knowledge of

the introduced genetic change to evaluate whether any nutritionally relevant pathways are likely to be significantly altered. If applicants have identified any such changes, the significance of the changes must be determined with reference to relevant peer reviewed research, as necessary.

Biological pathways (see Definitions) relevant to nutritional quality include those related to Bioaccumulation. Most biological transporters have more than one substrate. Applicants must consider how any change to a biological transporter affects any other substrate in addition to the target substrate.

For Example: An iron biofortified crop achieved by a modification of a trans-membrane transporter. Iron and zinc share a common transporter in many crops, and therefore the applicant must consider how the change effects zinc uptake as well as iron. If a significant change to zinc quantity in the plant is likely, the applicant should consider the potential hazards of increased zinc.

If the answer is No: This ends the safety assessment of Nutrition. Proceed to Section 11.

If the answer is Yes: Proceed to Step (**6.1**).

- **Step (6.1) – Is nutrient content likely to be significantly affected?**

  A significant alteration includes both increases and reductions in nutrient content that exceed the normal range found in TBO comparators that have a HSFU in the UK or EU, or beyond the ranges found in food composition dataset such as McCance and Widdowson's (2021).

  If the answer is No: Proceed to Step (**6.2**).

  If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. This ends the safety assessment of Nutrition. Proceed to Section 11 (Toxicity).

- **Step (6.2) – Is bioaccessibility likely to be significantly affected?**

  Bioavailability aims to describe the effect of metabolic events on nutrient utilization. The supply of nutrients to the human body depends not only on the amount of a nutrient in food but also on its bioavailability. The bioavailability of nutrients is highly variable and can be influenced by numerous factors. Different nutrients (including protein, iron, and vitamin A), and the forms in which they exist in the ingested medium, will react in different ways to inhibitors and enhancers as well as the host's nutritional status, all of which contribute to nutrient bioavailability.

  Bioaccessibility is the proportion of the nutrient that is available for absorption. A significant alteration includes both increases and reductions in

nutrient absorption. Bioaccessibility is affected by many factors (Schonfeldt, Pretorius, & Hall, 2016). Applicants must consider if any signal transduction pathways relevant to bioaccessibility, such as pathways likely to impact fibre, fat and protein content, plus antinutrient content, are affected.

If the answer is No: This ends the applicant's safety assessment of Nutrition. Proceed to Section 10.3.

If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition and a Regulation 22 application must be made. This ends the applicant's safety assessment of Nutrition. Proceed to Section 11 (Toxicity).

10.3. Conclusion of Tier 1 Safety Assessment for Nutrition

This ends the Tier 1 safety assessment of Nutrition. Where the answer to any question identifies a need for a Tier 2 safety assessment a Regulation 22 application must be made. Otherwise, no further safety assessment is required for Nutrition.

11. Toxicity Tier 1 safety assessment

11.1. Introduction to Toxicity

This part of the guidance specifically addresses the requirement in Regulation 20 (1) (c) (ii): "The applicant is able to demonstrate that the application of modern biotechnology does not introduce genetic changes that are expected to significantly elevate the toxicity of any food or feed produced from the precision bred organism."

Substances (i.e., elements, compounds and proteins) with a range of structures and chemical/biological functions can exhibit toxicity, impacting the health of human and animals consuming them as part of food and feed.

Substances of concern for toxicity in plants include natural toxins, and other chemicals that can exert toxic effects when their levels are significantly increased (well above normal ranges in plants for food/feed) resulting in abnormally high dietary exposure.

Proteins and/or metabolites with toxic effects can also be produced new to the organism. This can result from the introduction of new sequences or new enzymatic function, or from the activation of a normally silent pathway. The possible occurrence of these must be identified in Section 16.3 (Description of the genetic change(s)).

For the toxicity assessment, toxic substances do not include antinutritional factors: when substances reduce the bioavailability of nutrients by interfering with digestion and absorption of nutrients from food, their safety must be assessed in Section 10 (Nutrition). However, some substances (for example lectins) may demonstrate both toxic and anti-nutritional effects and must also be considered in the Toxicity section when relevant.

Where authorisation is sought for multiple PBOs as part of a batch (see Section 6.2), each question must be considered for all PBOs within the batch. Any difference in toxicity expected between the different PBOs within the batch must be clearly identified for each question.

11.1.1. Natural toxins

Naturally occurring toxins (hereafter referred to as natural toxins) are substances produced as part of the natural defence mechanism of the plant against predators, insects, microorganisms, or climate-related stress (World Health Organization, 2023). They are generally well characterised, and breeders will be aware of their presence within the organism.

Examples of natural toxins include, but are not limited to:

- **Toxic non-protein substances** such as: cyanogenic glycosides (for example, in sorghum, cassava and lima beans); furocoumarins; alkaloids including glycoalkaloids (for example, solanines, chaconine) and pyrrolizidine alkaloids (PA); and a variety of phytotoxins (for example, oxalates, resins, toxalbumins).
- **Toxic proteins** (specifically composed of amino acids), as reviewed by Kocyigit *et al.* (2023), include: Ribosome Inactivating Proteins (RIP, for example, saporin found in crops such as maize, barley); ureases; antimicrobial peptides (for example, thionins, cyclotides); and pore-forming toxins.

Natural toxins may be present in different parts of the plant (for example, leaves, fruits, roots, flowers), and their levels may be influenced by growth (particularly in response to stress) and post-harvest conditions. When natural toxins in a PBO are known to be a potential safety concern in food and feed, they must be considered in the applicant's safety assessment process to determine if their levels are safe. Where applicants are uncertain about the safety of the levels, the PBOs require a Tier 2 safety assessment.

While secondary metabolites (for example anthocyanins) are also produced by plants as part of protection mechanisms against abiotic stress, since they are not considered as natural toxins per se, they are treated as other substances (Section 11.1.2).

11.1.2. Toxicity from high level dietary exposure

Increased levels of substances in the plant, which can result in higher dietary exposure, may be intended as part of the genetic change(s) or may be reasonably anticipated as a consequence thereof (as identified in Section 16.3, Description of the genetic change(s)). Secondary effects of the genetic change(s) may also influence the bioaccumulation of toxic substances in the plant and must be considered.

Examples of mechanisms by which the genetic change(s) may indirectly or as a secondary effect increase levels of substances:

- Altered plant metabolism may indirectly significantly increase levels of related secondary metabolites that may be toxic in food or feed.
- Uptake and bioaccumulation of undesirable substances from soil or the environment (such as metals, organic pollutants, salts, nitrate, PAH, etc.) may be significantly increased alongside an improved uptake of nutrient intended by the genetic change(s).
- Significantly altered cultivation conditions linked to the genetic change(s) may significantly increase the accumulation of toxic compounds in the tissues of the plant.
- Significantly altered pre-harvest or post-harvest handling as a consequence of the genetic change(s) may result in increased attachment and persistence of microbiological contaminant(s).
- Increased resistance to pests linked to the genetic change(s) may involve the sequestration of toxic substances from other organisms or from the soil by the plant for defence purpose.

Applicants must understand which substances are reasonably anticipated to be increased in their PBO; however, where the levels remain in the range of those found in equivalent TBOs with a HSFU or a PFC, or within the range of relevant food composition datasets, this will not result in safety concern.

Variation in levels of substances in the PBO must be understood in order to characterise possible effects on dietary exposure, **considering existing Health-Based Guidance Values** (HBGVs, see Definitions) **as part of total intake** by humans (food) or by the target species (feed).

For most substances, toxicity will exhibit a threshold; however, applicants must consider the possibility of bioaccumulation of non-threshold toxic pollutants. When determining the significance of an increase, the levels must always be compared with HBGVs in the first instance; when those are not available, a Threshold of Toxicological Concern (TTC) approach, as described in the Guidance on the use of the TTC approach (EFSA Scientific Committee, 2019), may be appropriate.

While data on variations in levels of substances may not be required to be submitted for a Regulation 20 application, applicants are expected to obtain and retain it as a matter of due diligence in developing a holistic understanding of their PBO and maintain compliance with general obligations for ensuring the food and feed they produce is safe.

Levels of some of these substances are covered by existing regulations on maximum levels (Annex of assimilated Regulation 1881/2006 for food; Schedule 4 of Animal Feed (Composition, Marketing and Use) (England) Regulations 2015 for feed); these lists are regularly reviewed and amended to reflect the current knowledge in chemical risks from food and feed.

11.2. How to perform Tier 1 safety assessment for Toxicity

The Toxicity Tier 1 safety assessment requires answering the safety assessment question: "**Is the PBO designed to introduce changes that are expected to elevate significantly the toxicity of any food/feed derived from the organism?**" as described in Figure 5. This means addressing intended, reasonably anticipated and secondary effects. In navigating this safety assessment process, applicants must use the body of available scientific knowledge; databases of toxicity data on chemicals and tools for the prediction of the toxicity of chemicals may be sources of evidence. For major crop species, the OECD Consensus documents (OECD, live database) on plants provide a useful resource which list and describe the key toxicants. For species which are not covered, peer-reviewed scientific literature should be consulted to understand if natural toxins are present, or conversely if the targeted substance/protein is toxic.

As described in Sections 16.3.3 and 16.3.4 (Description of the genetic change(s)), only substances that are found in the edible tissues used for food/feed and that are either

new to the species, or above levels which have a relevant HSFU or PFC, may constitute safety concerns requiring safety assessment. Such substances should be identified as part of Steps (**1**) to (**4**), and be further safety assessed through Steps (**6**) to (**7**), unless applicants can demonstrate that the substance does not have any toxic effect at the levels expected to enter the food or feed chain.

To answer the question about Toxicity, levels of substances in the PBO should be compared to those in suitable reference databases (for example OECD consensus documents, McCann and Widdowson dataset) or in a suitable comparator. Different comparators may be selected for different purposes, and may include a TBO variety of the same species that has a HSFU/PFC, and shares the same role in the diet as the PBO (see Section 4).

Where the applicant's safety assessment identifies the presence of a substance at elevated levels that would warrant specific conditions of use that are new to the species and otherwise not already applied, a Tier 2 FSA safety assessment is required so that appropriate conditions of use can be determined (see Section 13.2.1).

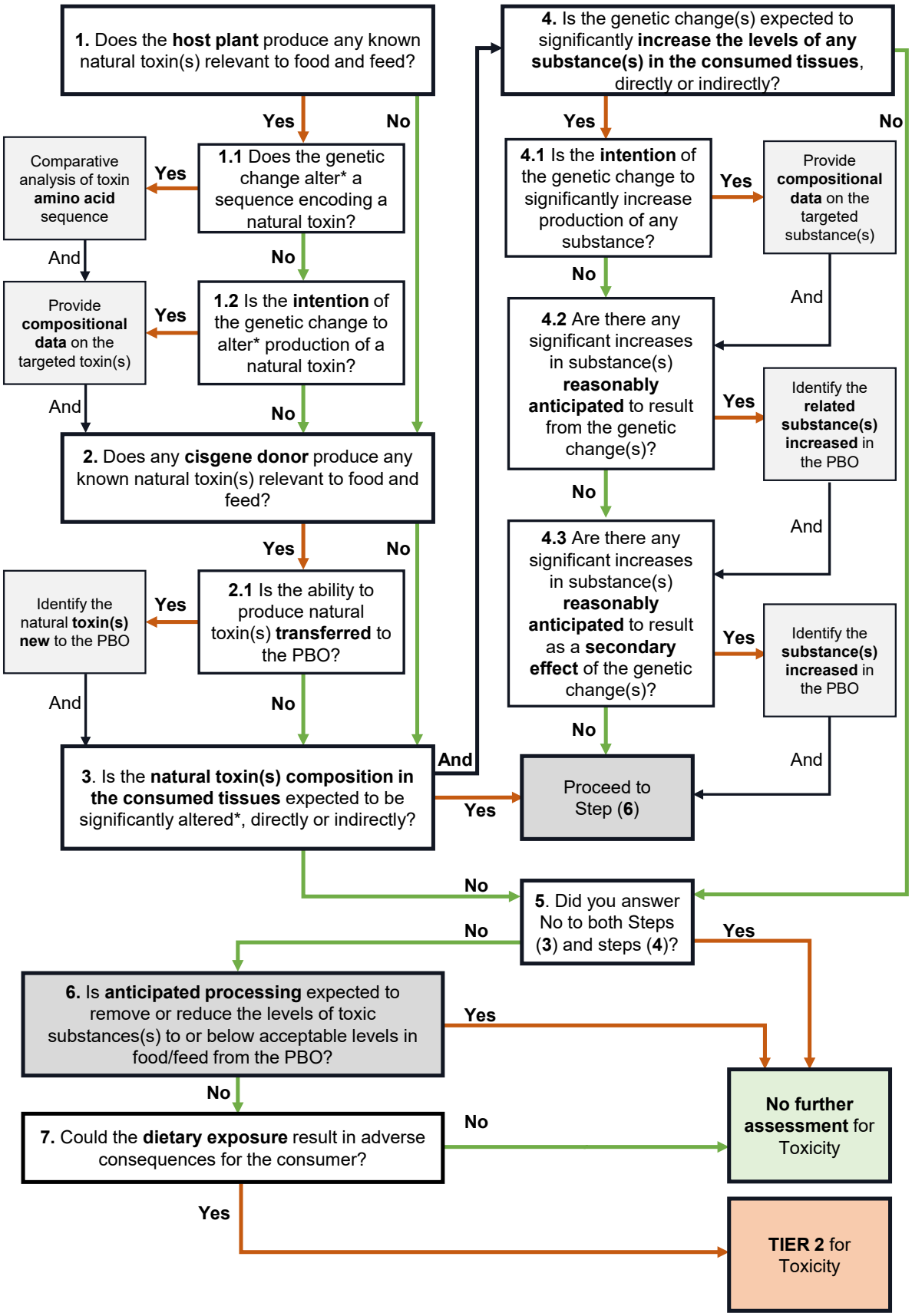


Figure 5.

Flowchart outlining the Tier 1 safety assessment process used to answer the safety assessment question about Toxicity: "**Is the PBO designed to introduce changes that are expected to elevate significantly the toxicity of any food/feed derived from the organism?**" A change is

significant if it is above existing Health-Based Guidance Values (HBGVs), or outside the ranges found in reference food composition datasets, or outside the ranges found in suitable comparators that have a HSFU or PFC, and is biologically relevant to safety. * Steps (**1.1**), (**1.2**) and (**3**), where there is a decrease in the production or activity of a natural toxin, consider the implications on how the PBO is consumed in Section 10.2 Step (**5**), and on how it is processed in Section 13.2.2. Where the Tier 1 safety assessment concludes that a Tier 2 safety assessment is required, a Regulation 22 application must be made. For detailed instructions, refer to Section 11.2.

11.2.1. Part A – Safety assessment for toxicity from natural toxins

Steps (**1**) and (**2**) identify **intended, direct effects** of the genetic change on the composition of natural toxin(s) in the plant. Step (**3**) identifies **reasonably anticipated effects**, as a result of the intended change or as a secondary effect from it.

Step (1) – Does the host plant produce natural toxin(s) relevant to food and feed?

Consider taxonomic information, peer reviewed scientific literature, public reference database (for example, OECD consensus documents on plants (live database), EFSA Compendium of Botanicals (2012)), proprietary compositional data and HSFU.

If the answer is No: Proceed to Step (**2**).

If the answer is Yes: Proceed to Step (**1.1**).

- **Step (1.1) – Does the genetic change alter a sequence encoding a natural toxin?**

  This step is relevant solely for protein toxins. Information to understand whether the gene targeted by the genetic change encodes a toxin includes sequence homology analysis (for example BLAST searches) with an available annotated database (for example GenBank, UniProt, String, EMBL-EBI), peer reviewed scientific literature or proprietary phenotypic or toxicology data. Alteration in the sequence of a natural toxin has the potential to increase its toxicity. Where the intent of the change in sequence is to decrease the toxicity of the natural toxins, the implications on how the PBO is consumed (Section 10.2, Step (**5**)) or how it is processed (Section 13.2.2) must be considered.

  If the answer is No: Proceed to Step (**1.2**).

  If the answer is Yes: Provide the conclusions of the analysis of the amino acid sequence alignments of the protein targeted by the genetic change for the PBO and the progenitor, and compositional data on the encoded toxin; proceed to Step (**2**).

- **Step (1.2) – Is the intention of the genetic change to alter production of a natural toxin?**

This would have been identified in Section 16.3 (Description of the genetic change(s)). Where levels of natural toxins are changed, only increases are a concern for the toxicity of food/feed. However, where the intention of the genetic change is to decrease the production of a natural toxin, the implications on how the PBO is consumed (Section 10.2, Step (**5**)) or how it is processed (Section 13.2.2) must be considered.

If the answer is No: Proceed to Step (**2**).

If the answer is Yes: Provide compositional data on the targeted toxin; proceed to Step (**2**).

Step (2) – Does any cisgene donor produce any known natural toxin(s) relevant to food and feed?

This can be answered using the same range of information as described in Step (**1**).

If the answer is No: Proceed to Step (**3**).

If the answer is Yes: Proceed to Step (**2.1**).

- **Step (2.1) – Is the ability to produce natural toxin(s) transferred to the PBO?**

  To understand if natural toxin(s) known to be produced by the donor plant are now produced by the host plant, the function of the DNA sequences transferred from the donor to the host species must be considered, as described in Section 16.3 (Description of the genetic change(s)).

  Where any substance(s) produced are new to the organism or to the diet, they must be included for consideration in Steps (**6**) and (**7**) of the Toxicity safety assessment. This is due to the absence of any HSFU or PFC of the PBO as a dietary source of these substance(s).

  If the answer is No: Proceed to Step (**3**).

  If the answer is Yes: Consider the natural toxin(s) newly produced in the PBO in Step (**6**) of the Toxicity safety assessment; proceed to Step (**3**).

Step (3) – Is the natural toxin(s) composition in the consumed tissues expected to be significantly altered, directly or indirectly?

Where levels of toxin(s) are increased above the ranges found in relevant comparator(s) or when a change in amino acid sequence of a toxin has the potential to significantly alter its potency, these are only relevant to the safety of food/feed when the affected toxins are produced, transported to, or deposited/sequestrated/stored in the edible tissues used for food/feed (Section 16.3.3 Description of the genetic change(s)).

While the focus of the safety assessment is on the intended effects of the genetic change(s), applicants are expected to have an understanding of the **additional**

anticipated direct effects, as described in Sections 16.3.2 and 16.3.4 (Description of the genetic change(s)). This also includes: the potential for the introduction of new genes (for cisgenesis/intragenesis) to contribute to existing biological pathways and restore/activate functions normally silent in the host; the potential for identified unintended, off-target changes (see Definitions) to the genetic material to interfere with the production or activity of toxin(s).

When they can be reasonably anticipated, the applicant's safety assessment must also take into account **secondary effects** of the genetic change on the levels of natural toxins produced in the plant (as described in Section 5). Natural toxins are frequently part of mechanisms of defence of the plant against biotic and abiotic stresses, therefore, they are particularly likely to be expressed at different levels depending on the growth conditions for the plant.

Information to support decision making on changes in the composition of natural toxins in the PBO includes the body of knowledge from peer reviewed scientific literature or proprietary phenotypic or toxicology data.

If the answer is Yes: Complete Steps (**4**) and (**6**).

If the answer is No: Complete Steps (**4**) and (**5**).

11.2.2. Part B – Safety assessment for toxicity from high level dietary exposure

Step (4) – Is the genetic change(s) expected to significantly increase the levels of any substance(s) in the consumed tissues, directly or indirectly?

Decision on this may be supported from the information required in Section 16.3 (Description of the genetic change(s)) and guided through Steps (**4.1**), (**4.2**) and (**4.3**).

These increases in levels of substance(s) are only relevant to the safety of food/feed when the affected substances are produced, transported to, or deposited/sequestrated/stored in the edible tissues used for food/feed (Section 16.3.3, Description of the genetic change(s)), and are expected to be above the ranges found in relevant comparator(s). Evidenced demonstration that such a substance does not exert toxicity by threshold may exempt it from consideration in Steps (**6**) and (**7**) of the Toxicity safety assessment.

If the answer is No: Proceed to Step (**5**).

If the answer is Yes: Proceed to Step (**4.1**).

- **Step (4.1) – Is the intention of the genetic change to significantly increase production of any substance?**

  Section 16.3.3 (Description of the genetic change(s)) identifies substances whose levels are intended to be increased in the PBO. Where the levels are

within ranges found in comparators for which a HSFU and a PFC exist, the changes are not considered significant. Where applicants can demonstrate that the substance does not have any toxic effect at the levels expected to enter the food or feed chain, the changes are not considered significant since they are not biologically relevant.

If the answer is No: Proceed to Step (**4.2**).

If the answer is Yes: Provide a summary of compositional data on the targeted substance(s) and consider the substance in Step (**6**) of the Toxicity safety assessment; proceed to Step (**4.2**). Samples must be obtained from organisms grown using conditions representative of those during food/feed growth. This may be contained growth or field conditions; stress-response traits will require presence of the stressor to be representative.

- **Step (4.2) – Are there any significant increases in substance(s) reasonably anticipated to result from the genetic change(s)?**

Step (**4.1**) identifies substances whose levels are intended to be increased in the PBO.

While the focus of the safety assessment is on the intended effects of the genetic change(s), applicants are expected to have an understanding of which **related substances** (see Definitions) may have significantly increased levels as an additional anticipated direct effect of the genetic change, as described in Sections 16.3.2 and 16.3.4 (Description of the genetic change(s)). The potential for the introduction of new genes (for cisgenesis/intragenesis) to contribute to existing biological pathways and restore/activate functions normally silent in the host must also be considered.

Information to support decision-making on changes in levels of substances in the PBO includes the body of knowledge from peer reviewed scientific literature or proprietary phenotypic data.

Where the levels are within ranges found in comparators for which a HSFU and a PFC exist, the changes are not considered significant. Where applicants can demonstrate that the substance does not have any toxic effect at the levels expected to enter the food or feed chain, the changes are not considered significant since they are not biologically relevant.

If the answer is No: Proceed to Step (**4.3**).

If the answer is Yes: Consider the related substance(s) increased in the PBO above ranges found in relevant comparator(s) in Step (**6**) of the Toxicity safety assessment; proceed to Step (**4.3**).

- **Step (4.3) – Are there any significant increases in substance(s) reasonably anticipated to result as a secondary effect of the genetic change(s)?**

When they can be reasonably anticipated, the applicant's safety assessment must also take into account secondary effects of the genetic change on the levels of substances produced, bioaccumulated or sequestered in the plant (as described in Sections 5 and 11.1.2).

Information to support decision-making on changes in levels of substances in the PBO includes body of knowledge from peer reviewed scientific literature or proprietary phenotypic data.

Where the levels are within ranges found in comparators for which a HSFU and a PFC exist, the changes are not considered significant. Where applicants can demonstrate that the substance does not have any toxic effect at the levels expected to enter the food or feed chain, the changes are not considered significant since they are not biologically relevant.

If the answer is No: Proceed to Step (**6**).

If the answer is Yes: Consider the related substance(s) increased in the PBO above ranges found in relevant comparator(s) in Step (**6**) of the Toxicity safety assessment; proceed to Step (**6**).

11.2.3. Part C – Mitigating factors for toxicity

Step (5) – Did you answer "no" to both Steps (3) and (4)?

If the answer is Yes: This ends the Tier 1 safety assessment of Toxicity, no further safety assessment is needed. Proceed to Tier 1 safety assessment of Allergenicity in Section 12.

If the answer is No: Proceed to Step (**6**).

Step (6) – Is anticipated processing expected to remove or reduce the levels of toxic substance(s) to or below acceptable levels in food/feed from the PBO?

Levels of toxic substances have the potential to be reduced through post-harvest processing.

Processing steps which alter the state of the food/feed product in such a way as to reduce absorption/alter disposition/increase excretion rather than by removing/destroying/inactivating the toxic substances do not provide sufficient reassurance on the safety outcome for the food/feed made of the PBO.

Levels of toxic substances are not considered reduced in processed food/feed when they are anticipated to remain above the levels found in food/feed from the progenitor of the PBO or from existing equivalent TBO crops after processing.

Information to support decision-making on this is an identification of processing steps (together with their efficacy) by which the toxin(s) or substance levels are managed through standard food-safety management systems used by anticipated processors

(for example, this may include heat treatment, extraction, distillation, squeezing, fractionation, purification, concentration, fermentation, or other procedure(s), or as described in the EFSA guidance for the assessment of detoxification processes in feed (2024)). This may be based on the body of knowledge from peer reviewed scientific literature or proprietary analytic data. Decision making must consider potential recent novel uses from whole, parts or extracts from organisms, and possible change in process allowed by the phenotypic change targeted by the PB.

If the answer is No: Proceed to Step (**7**).

If the answer is Yes: This ends the Tier 1 safety assessment of toxicity, no further safety assessment is needed for Toxicity. Proceed to Tier 1 safety assessment of Allergenicity in Section 12. To note, where the food/feed from the PBO need to be processed **differently** than food/feed from the progenitor to manage the levels of the substance, the Tier 2 safety assessment is required so that appropriate recommendations for conditions of use can be made (see Section 13.2.1).

Step (7) – Could the dietary exposure result in adverse consequences for the consumer?

Where levels of substance(s) or natural toxins are anticipated to remain above the levels found in food/feed from the progenitor of the PBO or from existing equivalent TBO crops after processing, it is judged significant when it is anticipated to result in high level dietary exposure. This requires an understanding of the anticipated levels of these substances in the plant and of the role in the diet of food/feed derived from it.

It is the responsibility of applicants to ensure that levels of any substance(s) comply with existing legal limits or are unlikely to cause harm (directly or by interacting with other substances in the food or feed).

Information to be used to support decision making on this **for food** includes: predictive or proprietary quantitative information on the levels in the PBO; body of knowledge and/or available peer-reviewed scientific literature; consumption databases such as the EFSA Comprehensive Food Consumption Database (2018) or the NDNS survey (Public Health England, 2020) to determine whether the PBO is a major part of the diet of any population.

Information to be used to support decision making on this **for feed** includes: Appendix C of the EFSA statement on the animal dietary exposure in the risk assessment of contaminants in feed (2024).

If the answer is Yes: The anticipated higher levels of dietary exposure for the identified substance(s) requires a Tier 2 FSA safety assessment for toxicity; a Regulation 22 application must be made. This ends the Tier 1 safety assessment of Toxicity. Proceed to Tier 1 safety assessment of Allergenicity in Section 12.

If the answer is No: This ends the Tier 1 safety assessment of toxicity, no further safety assessment is needed for Toxicity. Proceed to Tier 1 safety assessment of Allergenicity in Section 12.

12. Allergenicity Tier 1 safety assessment

12.1. Introduction to Allergenicity

This part of the guidance specifically addresses the requirement in Regulation 20 (1) (c) (iii): "The applicant is able to demonstrate that the application of modern biotechnology does not introduce genetic changes that are expected to alter the allergenicity of any food or feed produced from the precision bred organism."

Food allergy is defined as an adverse health effect arising from a specific immune-mediated response that occurs reproducibly upon oral exposure to a given food. Food allergies represent an important public health problem, and impact around 7.4% of adults in the UK (Simpson et al., 2024). Two types of immune-mediated adverse reaction have been clearly linked to food triggers: those mediated by Immunoglobulin E (IgE), and the T-cell mediated reaction known as Coeliac disease. The molecules involved in triggering food allergy are known as food allergens and are almost entirely proteins.

As with TB, genetic changes introduced through PB may alter pathways associated with allergen production in the plant. This may inadvertently alter endogenous allergenicity of the produced food/feed. The impacts may be predictable from knowledge of the gene function affecting allergen expression.

This section must be used to assess whether the introduced genetic change affects the levels of endogenous or intentionally introduced allergens in a manner that would adversely impact on human and animal health.

Where authorisation is sought for multiple PBOs as part of a batch (see Section 4.2), each question must be considered for all PBOs within the batch. Any difference in allergenicity expected between the different PBOs within the batch must be clearly identified for each question.

12.2. How to perform Tier 1 safety assessment for Allergenicity

The Allergenicity Tier 1 safety assessment requires answering the safety assessment question: **"Does the PB introduce changes that are expected to alter the allergenicity of any food/feed derived from the organism**?" as described in Figure 6.

This guidance document provides further information on:

- Organisms which are of allergenic concern;
- Relevant allergenic proteins;
- Methodology to be used for quantification of allergenicity, where relevant; and
- Principles to be followed for data submission.

Changes to allergens as a consequence of PB may increase allergenic risk. These allergens may originate within the PBO itself or within a closely related species from

which a gene is introgressed using cisgenesis. Applicants whose PBO does not involve an allergenic organism must still read and answer questions within this section, though a conclusion on allergenic safety will likely only require a Tier 1 safety assessment for Allergenicity.

The FSA requires two assurances for marketing of PBOs for consumption:

1. That there is no significant increase to the quantity of a known allergenic protein in the consumed tissues of a PBO which may increase allergens in the produced food/feed.

2. That if there is a decrease in, or removal of, an allergen for the purpose of consumption by an allergic population, any reduced allergenicity claim is substantiated.

To answer the safety assessment question for Allergenicity, historic allergenicity of the PB trait/organism should be compared to suitable reference databases or comparators. Different comparators may be selected for different purposes, and may include a TBO variety of the same species that has a HSFU/PFC and for which the potential to induce an allergenic response is understood.

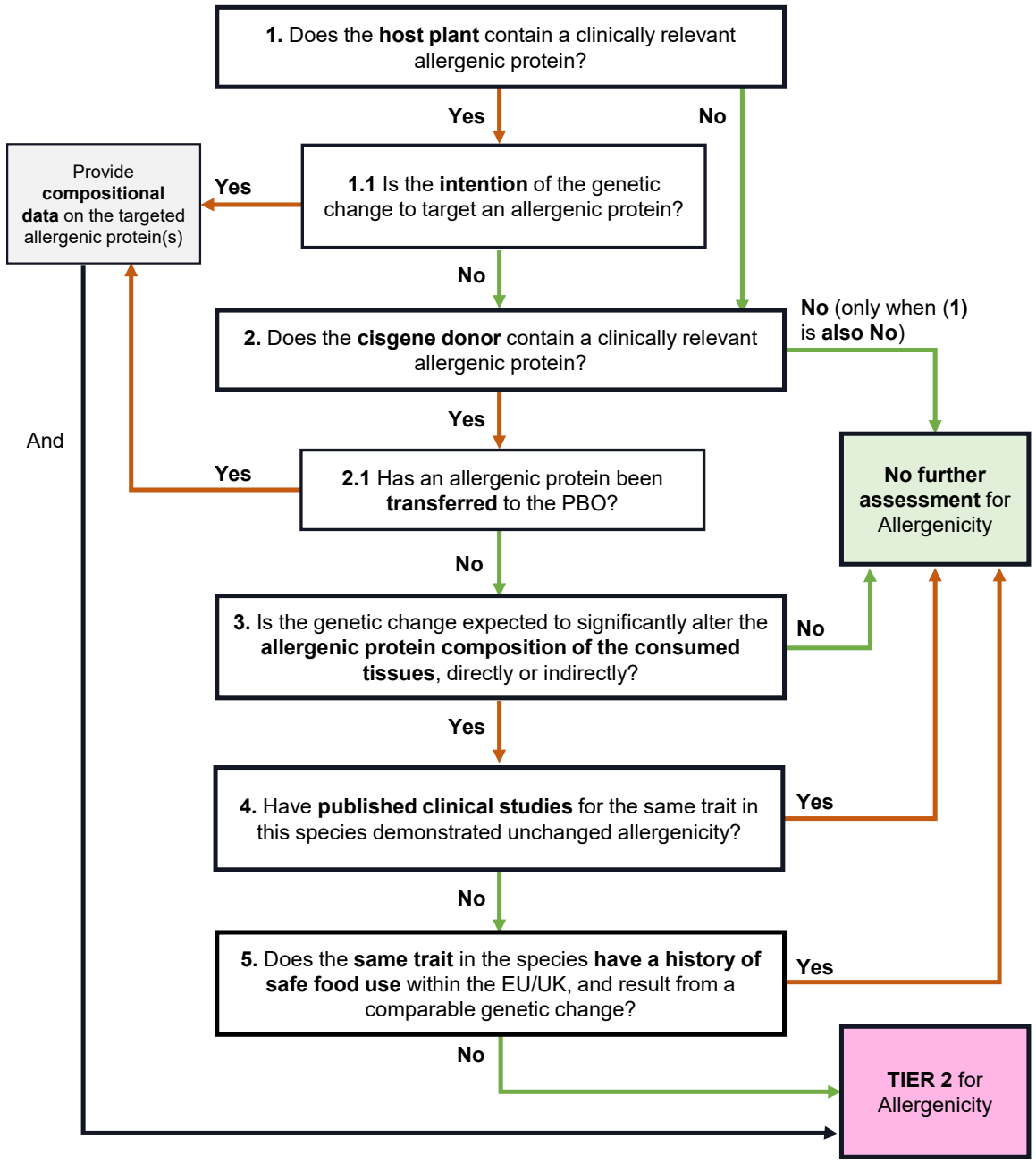


Figure 6.

Flowchart outlining the Tier 1 safety assessment process used to answer the safety assessment question about Allergenicity: "**Does the PB introduce changes that are expected to alter the allergenicity of any food/feed derived from the organism?**" A change in allergenicity is significant if it is outside the ranges found in reference food composition datasets by an order of magnitude, or outside the ranges found in suitable comparators that have a HSFU in the UK or EU by an order of magnitude, and is biologically relevant to safety. Where the Tier 1 safety assessment concludes that a Tier 2 safety assessment is required, a Regulation 22 application must be made. For detailed instructions, refer to Section 12.2.

Step (1) – Does the host plant contain a clinically relevant allergenic protein?

Organisms recognised to be common food allergens and of public health importance include those subject to mandatory labelling listed in Annex II of assimilated Regulation 1169/2011 on food information to consumers, and those with a high allergenic concern in the UK or EU. These are species for which scientific literature has established significant allergenic prevalence, potency, and severity. Clinically relevant allergenic organisms can be identified using the current literature, for example the Risk Assessment of Food Allergens, Part 1 (FAO & WHO, 2022a); EuroPrevall UK birth cohort (McBride et al., 2012); FSA Patterns and prevalence of adult food allergies (PAFA) (Simpson et al., 2024).

If the organism is not of allergenic concern, answer "No" to Step (**1**). If the PBO is a species of allergenic concern, answer "Yes" **to Step (1**). The target of the genetic change must be considered further in Step (**1.1**).

If the answer is No: Proceed directly to Step (**2**).

If the answer is Yes: Proceed to Step (**1.1**).

- **Step (1.1) - Is the intention of the genetic change to target an allergenic protein?**

  This would have been identified in Section 16.3.1 (Description of the genetic change(s)). Use **peer reviewed scientific literature** to determine if the protein(s) targeted by the genetic change is an endogenous clinically determined allergenic protein. **Databases** of allergenic proteins may be useful to consult for information on allergenic proteins.

  The following databases may be used to perform an alignment search of the nucleotide/amino acid sequence of the gene(s)/protein(s) targeted by the genetic change against clinically relevant allergens:

  http://www.allergenonline.org/ ; https://allergen.org/ ; http://www.allermatch.org/

  The name of the PBO and cisgene donor species (including common name) can also be searched within databases to generate a list of allergens they contain. These databases contain useful information on the allergens, such as the allergen name, corresponding gene/protein name, amino acid sequence, and links to external databases such as NCBI and GenBank Proteins/Nucleotides:

  https://db.comparedatabase.org/ ; https://www.allergome.org/ ; https://allergen.org/

  For species listed, applicants must compare the gene(s) impacted by the genetic change and ensure that it is not present within one of these databases, or within the scientific literature, as a clinically relevant allergen. Significant matches to endogenous allergens (including from the cisgene donor) **must be**

considered as a "Yes" to Step (1.1) and the PBO will require an additional Tier 2 safety assessment, and compositional data on the allergenic protein generated. Where the match is partial, scientific literature may be consulted to confirm the allergenicity of the protein prior to answering the question. If in doubt, answer "Yes" and the FSA will advise as part of the Tier 2 safety assessment data request.

For example, increasing expression of a gene directly involved in synthesis of an allergen, which may significantly increase the quantity of the allergen. This may alter the eliciting dose and increase the chance of an allergenic response when consuming the produced food.

If the answer is No: Proceed directly to Step (**2**).

If the answer to (1.1) is Yes: A Tier 2 safety assessment is required for allergenicity, and a Regulation 22 application must be made. In addition, quantitative compositional data of this protein will need to be generated, the details of which are outlined in Section 5.4. Continue to Step (**2**) to consider other concerns which may be raised due to changes in allergens.

Step (2) – If the PBO contains a cisgene, does the cisgene donor organism contain a clinically relevant allergenic protein?

This can be answered using the same range of information as described in Step (**1**).

For example, introgression of cisgenes from an allergenic species into a closely related but non-allergenic species may cause an allergic response when consumed.

If the answer is No AND the answer to Step (1) is No: This ends the Tier 1 safety assessment of Allergenicity, and no further assessment is required for allergenicity. Proceed to Tier 1 safety assessment of Other Safety Concerns in.

If the answer is Yes: Proceed to Step (**2.1**).

- **Step (2.1) Has an allergenic protein been transferred to the PBO?**

  To understand if allergens known to be produced by the donor plant are now produced by the host plant, the allergenic function of the DNA sequences transferred from the donor to the host species must be considered, as described in Section 16.3.1 (Description of the genetic change(s)). This can be answered using the same range of information as described in Step (**1.1**). Briefly, applicants should perform the relevant database searches and ensure the cisgene is not on the list of known allergens for that donor species.

  If the answer is No: Proceed to Step (**3**).

  If the answer is Yes: A Tier 2 safety assessment is required for Allergenicity, and a Regulation 22 application must be made. In addition, quantitative compositional data of this protein will need to be generated, the details of

which are outlined in Section 5.4. Continue to Step (**3**) to consider other concerns which may be raised due to changes in allergens.

Step (3) – Is the genetic change expected to significantly alter the allergenic protein composition of the consumed tissues, directly or indirectly?

Applicants are expected to have an understanding of the **additional anticipated direct effects**, as described in Sections 16.3.2 and 16.3.4 (Description of the genetic change(s)). This includes: the potential for the introduction of new genes (for cisgenesis/intragenesis) to integrate into existing biological pathways and restore/activate functions normally silent in the host; the potential for identified unintended, off-target changes to the genetic material to interfere with the production or activity of allergenic proteins.

When they can be reasonably anticipated, the safety assessment must also take into account **secondary effects** of the genetic change on the levels of allergens produced in the plant (as described in Section 5). Because allergenic proteins are frequently linked to stress-response, they are particularly likely to be expressed at different levels depending on the growth or storage conditions for the plant.

Impacts of the genetic change are only relevant to the safety of food/feed when impacting the allergenic proteins produced, transported to, or deposited/sequestrated/stored in the edible tissues used for food/feed (see Section 16.3.3, Description of the genetic change(s)). Increases in levels of allergens are significant if the quantity of protein in the edible tissue is expected to be above the ranges found in equivalent TBOs by an order of magnitude (Houben et al., 2020).

Information to support decision making on changes in the composition of allergens in the PBO includes the body of knowledge from peer reviewed scientific literature or proprietary phenotypic data.

For example, changes to a trait confined to leaf tissue will not be relevant to allergenicity if only the fruit is consumed.

If the answer is No: This ends the Tier 1 safety assessment of Allergenicity, and no further assessment is required for allergenicity. Proceed to Tier 1 safety assessment of Other Safety Concerns in Section 13.

If the answer is Yes: Proceed to Step (**4**).

Step (4) – Have published clinical studies for the same trait in this organism demonstrated unchanged allergenicity?

Applicants must identify a peer reviewed, published scientific study which conducted an oral challenge for the same phenotype which has originated from a functionally equivalent genetic change. The study must show that when the organism is consumed

in the same form(s) intended for the PBO, no increase in allergenic response is observed. Applicants must provide a reference to this study/studies.

If the answer is Yes: This ends the Tier 1 safety assessment of Allergenicity, and no further assessment is required for allergenicity. Proceed to Tier 1 safety assessment of Other Safety Concerns in Section 13.

If the answer is No: Proceed to Step (**5**).

Step (5) – Does the same trait in the species have a history of safe food use within the EU/UK, and result from a comparable genetic change?

This question is intended for PBOs where the genetic change has been made to generate a genomic sequence which is the same as a traditionally bred variety already on the market.

For example: to minimise linkage drag, such as obtaining a desirable trait present within an exotic variety within an elite variety.
For example: to introgress a pathogen resistance receptor from an older crop variety to confer disease resistance within an elite variety.

Information to be used to support decision making on this includes: comparative analysis of the genomic sequences of the PBO and the species already on the market demonstrating the similarity of the genetic change; and, a body of knowledge and/or available peer-reviewed scientific literature demonstrating HSFU of the species in the EU/UK with the comparable genetic change. This would have been identified in Section 16.3 (Description of the genetic change(s)).

The genetic sequence must be within the primary gene pool of the PBO. Applicants must have evidence that the genotype and the trait of the comparator has a reasonable HSFU to answer this question.

If the answer is Yes: This ends the Tier 1 safety assessment of Allergenicity, and no further assessment is required for allergenicity. Proceed to Tier 1 safety assessment of Other Safety Concerns in Section 13.

If the answer is No: A Tier 2 safety assessment is required for Allergenicity, and a Regulation 22 application must be made.

13. Other Safety Concerns

13.1. Introduction to Other Safety Concerns

This part of the guidance specifically addresses the requirement in Regulation 20 (1) (c) (iv): "The applicant is able to demonstrate that the application of modern biotechnology to the PBO does not introduce genetic changes that are expected to introduce any additional features that may affect the safety of any food or feed produced from the PBO."

The 'other safety concerns' criterion requires applicants to identify potential hazards which are not of a compositional nature and those which might be the result of unforeseen use of the technology. Concerns to declare in the "Other Safety Concerns" category are any traits which could cause significant physical, physiological, or psychological harm, and which is not already covered under compositional sections.

When conducting a Tier 1 safety assessment for other safety concerns, applicants must apply their knowledge of the PBO to consider how any introduced traits, or altered processing or uses may impact safety in ways not covered by compositional assessment as performed in Sections 9 (Novelty), 10 (Nutrition), 11 (Toxicity) and 12 (Allergenicity). Likewise, applicants must clearly identify any gaps in methodology, or knowledge that may limit their ability to accurately identify safety concerns. When in doubt, applicants are advised to submit a Regulation 22 application. In such cases, both applicants and consumers will benefit from the assurance afforded by an independent third party assessment of safety.

Under Regulation 33 (1), market authorisations may be revoked or varied if there is new information which might affect the conclusions of the safety assessment of the PBO for use in food and feed.

If the answer to any question in Section 13.2 is yes, a Tier 2 FSA safety assessment is required and a Regulation 22 application must be made.

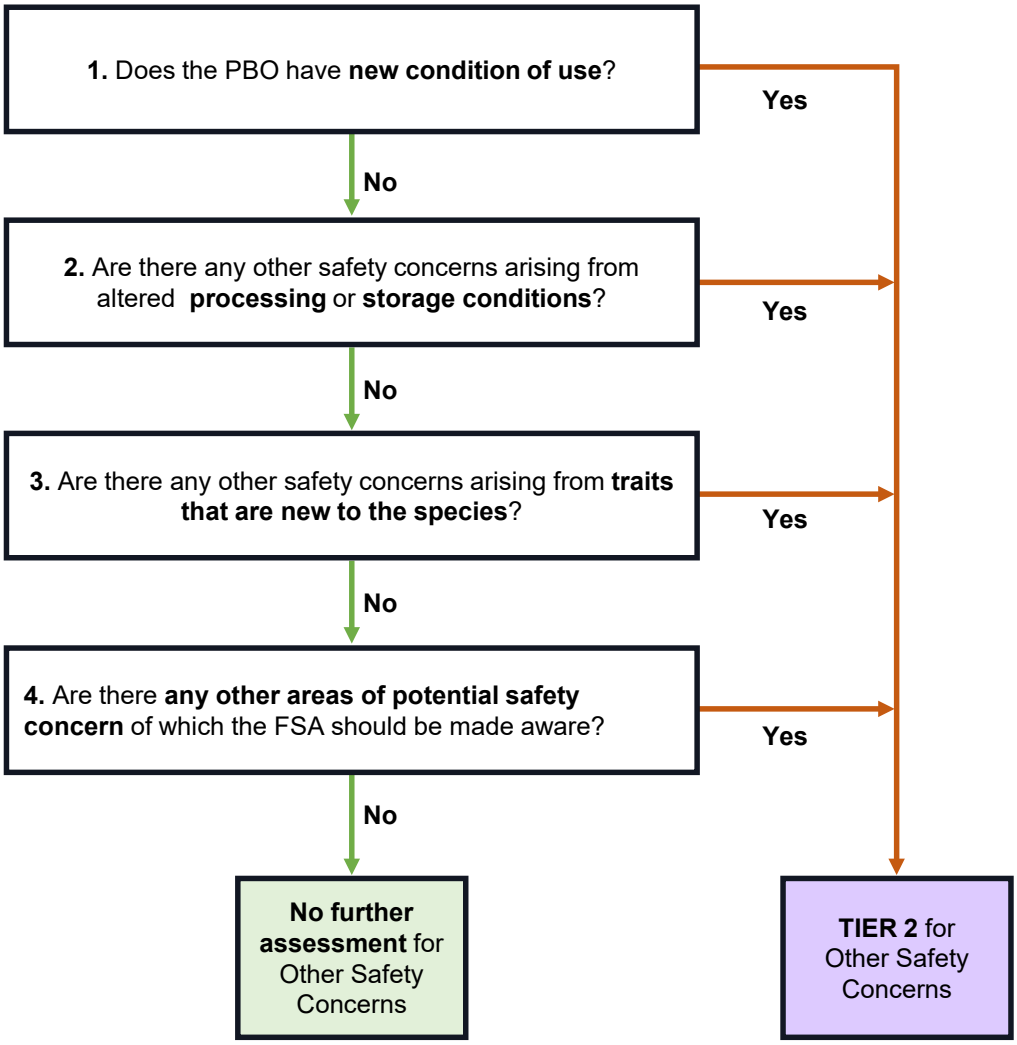


Figure 7.
Flowchart outlining the safety considerations used to answer the safety assessment question about other safety concerns: "**Are there any additional features of the PBO that cause food/feed safety concerns?**" It identifies a non-exhaustive list of features that may give rise to non-compositional safety issues that must be addressed in the considerations to answer the question about Other Safety Concerns. Where the Tier 1 safety assessment concludes that a Tier 2 safety assessment is required, a Regulation 22 application must be made. For detailed instructions, refer to Section 13.2.

13.2. Safety considerations for Tier 1 safety assessment of Other Safety Concerns

13.2.1. Does the PBO have a new condition of use?

The Secretary of State must consider whether a new marketing authorisation should be subject to any conditions or limitations under Regulation 30 (2).

New conditions of use may include any restrictions on the parts of the organism permitted for use in food or in feed, or restrictions on products which may be derived

from the PBO and at what quantities for safe use and have not been historically associated with the organism species. If recommendations for new conditions of use prior to authorisation are required, applicants must apply under Regulation 22.

Where a PBO is for **Feed use only,** a Regulation 22 application must be made so that appropriate recommendations for conditions of use can be made to avoid the PBO entering the food chain (for example, feed labelling).

Under Regulation 32 (3) (a) and (b), authorisation-holders (and other persons placing, or proposing to place, authorised food and feed on the market) must advise the FSA of any change in circumstances that may affect the safe use of the PBO in food or feed. This would include situations where they became aware that new or modified conditions or limitations may be required in respect of the authorisation. The FSA must also be informed prior to the application of any subsequent production process post authorisation, which would result in a food which would otherwise be considered novel under the novel food regulations. Where the Secretary of State becomes aware of such change(s), in accordance with Regulation 33, they may vary or revoke the authorisation.

If market authorisation for the food and feed from the PBO is subject to any conditions or limitations, these will also apply to any qualifying progeny under Regulation 19 (4). Conditions of use may prohibit the qualifying progeny of the PBO from combinations with traits that may cause safety concerns.

13.2.2. Are there any Other Safety Concerns arising from altered processing or storage?

If the PBO will be processed in a way that differs from conventional practices and may raise safety concerns, it requires an FSA Tier 2 safety assessment so that recommendations for appropriate conditions of use can be made.

Does the genetic change intentionally alter, or could be reasonably anticipated to alter, processing or storage conditions impacting key food safety measures, for example microbiological control measures?

Where the **intention of the change is to alter processing conditions**, or where it can be reasonably anticipated that **a processing step will be altered,** applicants are expected to have sufficient knowledge of the process in order to consider the impact of the alteration on food safety.

For Example:

Decreasing spoilage for extended storage. Applicant must evaluate the possible impacts of significantly longer storage times on potential safety concerns relating to chemical safety, e.g. accumulation of secondary metabolites.

Potential microbiological hazard. Significantly altered pre-harvest or post-harvest handling as a consequence of the genetic change(s) may result in increased attachment and persistence of microbiological contaminant(s).

Is a novel process intended to be used in conjunction with the genetic change to produce an intended compositional or structural trait within a food?

For a definition of a novel process, see Section 9.2, Step (**2**).

Some PBOs may require use of a specific processing step to fully achieve the intended trait (for example UV treatment); other traits may be introduced specifically to allow the PBO or a part of it to be processed using a new technique.

For Example:
- A plant which produces a precursor activated by UV light treatment to produce a nutritionally significant compound which is the intended benefit of the PBO. In this case, UV treatment constitutes a novel process.
- Change in cell wall composition specifically introduced to allow protein extraction via a novel extraction technology. In this case, the extraction technology constitutes a novel process.

13.2.3. Are there any Other Safety Concerns arising from traits that are new to the species?

Are there any changes in the physical morphology that may pose a choking, abrasive, puncture, or other mechanical hazard to the consumer?

For Example: A change in the physical morphology of the PB to introduce thorns or stinging trichomes. Consumption may cause physical harm to the consumer. The applicant may wish to discuss how this could be mitigated, such as a label to consume the PB cooked which would remove trichomes.

Are there similar combinations of traits in related species that are known to be harmful?

13.2.4. Are there any other areas of potential safety concern of which the FSA must be made aware?

Are there any gaps in knowledge or methodological uncertainties that significantly hinder an accurate safety assessment?

Applicants are expected to have sufficient background knowledge of both the host organism and the genetic change to identify safety concerns based on the available literature.

Is there any other scientific reason to believe the product may present safety concerns, based on the available knowledge of the trait(s), species and mechanism of action?

13.2.5. Where no Other Safety Concerns are identified

If the answer to all questions in Section 13.2 is no, and to the best of the applicants' knowledge there are no features of the PBO that give rise to any other safety concern

not covered in Part 2: This ends the Tier 1 safety assessment of Other Safety Concerns. No further safety assessment is required for Other Safety Concerns. Applicants must certify that to the best of their knowledge, the PBO does not present any other safety concerns.

If the answer to any question in Section 13.2 **is yes**: A Tier 2 safety assessment is required and a Regulation 22 application must be made.

14. Information to be provided following Tier 1 safety assessment

Part 3 identifies the information to be provided for all criteria for both Regulation 20 and Regulation 22 applications. Part 4 identifies the additional information that needs to be provided specifically for Regulation 22 applications, only for the criteria where the need for a Tier 2 safety assessment was identified during Tier 1 safety assessment.

Part 3 - Information to include in all applications

15. Information from Tier 1 safety assessment to include in both Regulation 20 and Regulation 22 applications

This section identifies information to include to satisfy Part (5) of Schedule 4 which states that applicants must provide "statements to demonstrate how the applicant has reached the conclusions in relation to the precision bred organism for each of the criteria set out in paragraphs (1) (b) and (c) of Regulation 20 including accompanying descriptive text setting out the applicant's key considerations and justification in respect of each criterion".

There are two regulatory routes to the authorisation of food or feed produced from a PBO following the applicant-led Tier 1 safety assessment: submission of a Regulation 20 application, or submission of a more detailed Regulation 22 application for Tier 2 FSA safety assessment.

Applicants should use their answers from the Tier 1 safety assessment to provide descriptive confirmation of the sources of evidence used when submitting an application. For batch applications, applicants should highlight where there are different answers for PBOs within the same batch. Detail on the types and sources of data to be used are detailed in the corresponding sections of the guidance. Datasets, including sequence data, are not required to be provided in a Regulation 20 application, although the FSA has the discretion to request any further information, including datasets referred to by applicants in their application, as part of verification under Regulation 24. Should the information requested not be provided in the time period specified by the FSA, the application will be treated as withdrawn. The FSA recommends authorisation holders retain sufficient records of any data used to perform their safety assessment and to reach conclusions as presented in their application, as these may also be requested in support of considerations of revocation or variation by the Secretary of States, in accordance with Regulation 33.

The information required for a Regulation 20 application also needs to be provided as the starting point for a Regulation 22 application.

16. Identity of the PBO and description of the genetic change(s)

16.1. Introduction to Identity and description of the genetic change(s)

The ability to assess potential risk to consumers and animals from the consumption of PBOs requires information describing the organism, the changes to expected use/exposure, and the potential safety concerns. Information on the identity and characteristics pertinent to the identification of the specific PBO must therefore be provided.

In navigating the safety assessment process, it is necessary to describe the genetic change(s), and to understand how the resulting phenotype will compare to that of a traditionally bred counterpart. Sources of evidence to be used include peer reviewed scientific literature, and a range of online databases such as GenBank, UniProt, String, EMBL-EBI, Reactome; other sources of evidence can be used where scientifically justified.

Where limited or no functional information is available for endogenous genes, information on the function of any homologue(s) in other species may be used from the closest available model organism with an annotated genome (for example, TAIR, or the Rice genome hub).

Where the function of an affected gene or the role of an increased substance for nutritional quality/safety of food/feed is unknown, this must be considered when addressing each safety assessment question on composition.

Where an applicant relies upon their own commercially sensitive annotated genome as evidence, the genomic data must still be provided to the FSA where required to demonstrate safety, but applicants can request for these to be treated as commercially confidential (Regulation 34).

The starting point in performing Tier 1 safety assessment is to clearly describe the purpose of the genetic change(s) and the reasons for targeting a specific alteration to the organism's genetic material. This enables identification of the **intended** and any **reasonably anticipated** effects (see Definitions) of the genetic change(s). The description of the genetic change(s) and of their resulting phenotype, and how the genetic change(s) compares to other genotypes and varieties of the same species support identifying whether a further Tier 2 FSA safety assessment is required for a PBO.

Information already required in the Marketing Notice to the Defra Secretary of State (Defra Marketing Notice) as described in Schedule 2 (3), (4) and (5) may be submitted

to the FSA where requirements overlap. However, **additional information specifically relevant to food/feed use** is also required for the application to the FSA for a food and feed marketing authorisation.

16.2. Information on Identity to include in all applications

Sections 16.2.1 to 16.2.4 specifically address the requirement in Schedule 4 (3). Section 16.2.5 identifies essential information to evidence conclusions on compositional criteria, in support of the requirement in Schedule 4 (4).

The details listed in Sections 16.2.1 to 16.2.5 **must** be provided for characterising the identity of the PBO.

16.2.1. Name of the PBO

- The unique reference number (URN) by which the PBO will be listed in Defra registry for authorised precision bred organisms.

Where the application is a **batch application** for multiple PBOs:

- How many PBOs are included in the batch, the URN for the batch and individual identifiers.

16.2.2. Taxonomic information

Information already required as part of the Defra Marketing Notice (Schedule 3 (1)):

- Taxonomic information allowing the identification of the PBO: Scientific (Latin) name including genus, species, according to the international codes of nomenclature.

The compositional profile relevant to the safety and quality of food/feed may vary significantly between the subspecies and varieties of a same species. Therefore, a same genetic change introduced into different subspecies or varieties may result in different safety profiles.

Additional information specifically for the FSA food and feed marketing authorisation application:

Where applicable:

- Subspecies or variety, according to the international codes of nomenclature.

Identifying subspecies or varieties is applicable when, for example:
- The subspecies or variety is biofortified;
- The subspecies or variety is pest-resistant and produces compounds absent in other subspecies or varieties;
- The allergenicity profile of the particular subspecies or variety is different from other subspecies or varieties.

Where the application is a **batch application** for multiple PBOs, where applicable and where they vary:

- Subspecies or variety should be specified for each PBO.

16.2.3. Purpose of the change

Information already required as part of the Defra Marketing Notice (Schedule 3 (2)):

- Brief description of the PBO and the purpose of the altered/introduced trait.

Additional information specifically for the FSA food and feed marketing authorisation application:

- Further detail on the purpose of the change related to food or feed should be given where relevant.

For example, to improve production/yield, biofortification for increased nutritional impact on human/animal diet, alteration of post-harvest handling/processing, improved biotic or abiotic stress tolerance, *etc*.

16.2.4. Intended use in food and feed

Information already required as part of the Defra Marketing Notice (Schedule 2 (4)):

- Brief description of the achieved trait, including: any new intended use likely to be adopted as a result of the organism's altered characteristic(s); whether the PBO is intended to replace another source of food or feed.

Additional information specifically for the FSA food and feed marketing authorisation application (Schedule 4 (1) (4) (c):

Where only specific parts of the organism are used for **food**:

- The part(s) intended for food use, for example, root, leaf, seed, *etc*., and whether they are affected by the change introduced by PB.

Where the PBO is used for **feed**:

- The part(s) intended for feed use or that may enter the feed chain, for example, root, leaf, seed, etc., and whether they are affected by the change introduced by PB – note these may be different from the parts intended for food use; for each part, state the animal species the feed is intended for.

Where the PBO is intended to be used exclusively in feed, this must be reported in Other Safety Concerns (see Section 13.2.1).

Where **conditions of use** that are new to the species are identified for a PBO for food or feed use:

- Brief description of the new condition(s) of use;
- How they may appear on labelling (for example, restricting the population of consumers or the intake per day).

Conditions of use must be reported in Other Safety Concerns (see Section 13.2.1).

16.2.5. Intended phenotype and rationale for targeting the specific genomic region

The reasons for targeting the specific gene/function in the organism must be provided in the form of a brief description / list.

Information already required as part of the Defra Marketing Notice (Schedule 2 (5) (e)) and associated technical guidance [insert reference when available]:

- What the effect of the introduced change is at the molecular level: for example, partial or complete loss of function of the gene, alteration of the properties of the encoded gene product, altered level of expression of the gene, gain of biological function, etc;
- What the intended trait and the intended impact of the genetic change on the characteristics (phenotype, including general effects on the physiology) of the organism are;
- Why the trait was obtained in this particular way, including reasoning for the choice of the target.

Factors that could have contributed to the targeting strategy might include but are not limited to: the target being known to have no or limited widespread effect; the target being known to combine multiple effects of interest; the specific copy number of the target favouring a positive outcome; the absence of known detrimental physiological consequences; the existing knowledge on the gene, its product, its function and cellular mechanism.

16.3. Description of the genetic change(s) to include in all applications

All submissions are required to contain sufficient detail on what genetic change(s) were made, how, for what reason, and what are the intended and reasonably anticipated consequences for the composition of the PBO. These represent key considerations and justifications in support of the information detailed in Schedule 4 (1) (5). The focus of the assessment should be the intended phenotype and how the genetic change(s) contributes to it; applicants are expected to have data and a good understanding of both. However, where additional unintended effects (see Definitions) relevant to the nutritional quality/safety of food/feed can be reasonably anticipated from the data available on the genetic change(s), they must also be identified.

Information to be used to understand the **function** of sequence(s) of an entire gene, or segments within a gene, directly affected by the genetic change in the host organism may include:

- Peer reviewed literature (including annotated sequences available in the public domain);

- Proprietary data (for example phenotypic comparison of the PBO and its progenitor); or
- Sequence homology analysis (sequence alignments (for example BLAST searches) with an available annotated database (for example GenBank, UniProt, String, EMBL-EBI).

Where information is not available, it must be clearly specified, and brief reasoning for why the applicant considers that it does not raise concerns must be provided.

See Section 15 for further instruction on the information to provide in all applications.

The details listed in Sections 16.3.1 to 16.3.4 **must** be provided as relevant.

16.3.1. The characteristics of the genomic change(s)

The characterisation of the change(s) of genomic features at the site of the change or of the insertion of a cisgene/intragene must be briefly described; this information is expected to have been obtained during the development stages of the PBO. The following information is required to perform the safety assessment of PBOs for food/feed use:

For small, targeted changes in sequence:

Information already required as part of the Defra Marketing Notice (Schedule 2 (5)) and associated technical guidance [insert reference when available]:

- Gene(s) name(s) and alternative name(s) (if in coding sequence);
- Primary function or hypothetical function of the coding sequence targeted, i.e. the properties or function of the product; whether the same locus on both strands holds different functions must be considered;
- Primary function or hypothetical function (if any) of the non-transcribed sequence targeted; whether the same locus on both strands holds different functions must be considered;
- Gene type, for example, whether it encodes a protein or is transcribed into non-coding RNA; whether the same locus on both strands holds different functions must be considered.

Additional information specifically for the FSA food and feed marketing authorisation application:

- Where multiple copies of the target sequence exist in the genome, whether all copies were altered; this may affect the intensity of the resulting phenotype.

For cisgenesis and intragenesis:

Information already required as part of the Defra Marketing Notice (Schedule 2 (5)) and associated technical guidance [insert reference when available]:

- For **cisgenesis**, detail of the genetic components introduced, i.e. on regulatory sequences and regulatory elements, coding sequences (gene(s) name(s) and

alternative name(s); primary function or hypothetical function; gene type); how many copies were introduced;

- For **intragenesis**, for each genetic component inserted: description of the elements within the inserted DNA fragment, i.e. regulatory sequences and regulatory elements, coding sequences (gene(s) name(s) and alternative name(s); primary function or hypothetical function; gene type); relevant information about the rationale for selecting the specific combination; how many copies were introduced;
- For each genetic component inserted: donor organism species and/or subspecies.

Additional information specifically for the FSA food and feed marketing authorisation application:

- Clear identification of: any metabolic function new to the plant; the phenotype they result in, which existed in cross-compatible species but were not normally present in the host plant, and whether they have a HSFU/PFC;
- Where reasonably anticipated, clear identification of: gene(s) normally silent in the plant which are now expressed; the substance(s) this allows the production of, and whether they have a HSFU/PFC;
- Where reasonably anticipated, clear identification of: gene(s) normally expressed in the plant which are now silent or which expression is reduced; the substance(s) this allows the production of, and what their role in the diet is.

Where any substance(s) produced as a result of the change are **new to an organism** commonly consumed or are **not normally found in food or feed**, these must be identified and documented with compositional information in Sections 10.2, 11.2 and 12.2, as relevant. This is due to the absence of any HSFU/PFC of the PBO as a dietary source of these substance(s).

On the location(s) and size(s) of the change(s) / insertion(s):

Information already required as part of the Defra Marketing Notice (Schedule 2 (5)) and associated technical guidance [insert reference when available]:

- Whether it is in the nuclear genome OR in non-nuclear genomes;
- Size of the alteration: number of nucleotides altered, deleted or inserted;

Additional information specifically for the FSA food and feed marketing authorisation application:

- Where the genetic change(s) is in a coding sequence: identification of the specific exon or intron targeted; how this affects the amino acid sequence where relevant;
- Where the genetic change(s) is in non-coding genetic material: applicants must have analysed sufficient flanking sequence such that the location of the insertion can be determined by comparison to a suitable reference sequence

if requested; identification of the closest coding sequences and their functions on both sides; where non-random insertion is used, relevant information about the rationale for selecting the specific site;

- Where the genetic change(s) is the result of cisgenesis or intragenesis: orientation of the insertion;
- Any identified undesired on-target event occurring during precision breeding and present in the final PBO must be described, together with its reasonably anticipated consequences on the nutritional quality/safety of food/feed.

16.3.2. Controls ("on-targets", "off-targets" and vector-derived sequences)

Where unintended editing events have been identified in the PBO in the Defra Marketing Notice (Schedule 2 (6) and (7)) and associated technical guidance [insert reference when available], provide:

- Description of the unintended events (location in the genome, the function they might affect, and relevance to the nutritional quality/safety of food/feed). Those alterations predicted to be relevant to the nutritional quality of food/feed must be taken into consideration for Tier 1 safety assessment of Nutrition (10), Toxicity (11) or Allergenicity (12).

16.3.3. The relevance of the intended change(s) for the safety and the nutritional quality of food/feed from the PBO

An intended change(s) is not relevant to the safety or nutritional quality of food/feed when it does not affect parts of the plants that are consumed as food or feed.

Information in this section is specifically required for the FSA food and feed marketing authorisation application.

On the distribution of the expression of the new phenotype in the parts of the organism:

- **Identification of the parts of the organism where the genetic change(s) is expected to result in the expression of a new phenotype**, due to the local expression of the targeted gene/function: this must be informed by available proprietary data and peer reviewed scientific literature**;**

In addition, the following should be considered and should be reported where relevant to food and feed use:

- **Identification of known moonlighting** (see Definitions) **of the gene**, where it is expressed for an alternative function in different tissues of the organism: this must be informed by available proprietary data and/or peer reviewed scientific literature;
- **Identification of transportation mechanisms** which distribute the phenotype across different tissues of the organism (including in locations where the

targeted gene/function is not expressed), and any resulting compositional changes.

Where the **purpose** of the genetic change(s) is to intentionally alter the composition of the PBO relevant to the safety/nutritional quality of food/feed made of it, provide:

- **Identification of the target substance(s) whose levels are intended to be changed:** expected changes in levels are identified by comparison to the levels in the progenitor genotype to demonstrate that the desired phenotypic change has been achieved. Where levels of a substance relevant to the nutritional quality or safety of a PBO are **intended** to be changed, compositional data on this substance must be reviewed as part of the safety assessment and be provided as described in Section 16.4.2.
- **Identification of whether the substance(s) whose levels are intended to be affected are relevant for the nutritional quality/safety of food/feed:** this can be identified based on available knowledge (for example peer reviewed scientific literature, databases such as those referenced in Sections 10 (Nutrition), 11 (Toxicity) and 12 (Allergenicity), or proprietary compositional analysis). For example: a decrease or an increase in the concentration of a nutrient is likely to affect nutrition; an increase may also affect toxicity; changing the chemical profile of an organism to repel or harm pest insects (antixenosis, antibiosis) could affect toxicity and/or allergenicity; reducing the levels of a known allergen must be examined for impact on allergenicity.
- **Brief description of the mechanisms by which the genetic change(s) alter the levels of the target substance(s) relevant to the nutritional quality/safety of food/feed:** changes to the characteristics of the protein encoded by a gene or changes to the expression of specific gene(s) may either directly impact the composition, or it may interfere with a biological pathway (for example, regulatory network, metabolic pathway, signal transduction pathway) and repress/induce the expression of other genes, affect catabolism/metabolism, transportation and availability of substances. Connection(s) to biological pathway(s) may be informed by published or proprietary data from proteomic, metabolomic, transcriptomic, or online databases (for example Plant Reactome, KEGG Pathway, TAIR, Rice Genome Hub).

16.3.4. Additional anticipated effect(s) from connection(s) to biological pathway(s)

While the focus of the safety assessment is on the intended effects of the genetic change(s), applicants are expected to have an understanding of the consequences of altering a step in a biological pathway. Whether intended or reasonably anticipated, such a change has the potential to affect the nutritional quality/safety of food/feed through changes in the expression of multiple genes and/or the production of multiple related substances. This may be the result of regulation of the expression of

genes (presence/absence of proteins/enzymes), disruption of signalling, competitive / non-competitive and feedback inhibition of enzymes (activity of proteins/enzymes).

Information in this section is specifically required for the FSA food and feed marketing authorisation application.

Where they can be reasonably anticipated:

- Identification of the **related substance(s)** (i.e., elements, compounds, proteins) relevant to the nutritional quality/safety of food/feed whose levels are indirectly significantly affected by the genetic change, and a brief description of the mechanisms leading to the changes in levels. This may be inferred from genetic and/or physiological knowledge, and/or published literature or proprietary data informing the expression of genes or proteins, or measurements of the substances they control the production of;
- For each relevant related substance identified: whether its level is expected to be significantly increased or decreased.

Where no links with any affected biological pathway relevant to the nutritional quality/safety of food/feed exist:

- **Confirmation** of the absence of anticipated effects on the composition relevant to the nutritional quality/safety of food/feed.

For example:

- Actors in a pathway may be a regulatory protein, a target gene, an enzyme, a component of signal transduction.
- Interactions may be repression, induction, activation or inactivation, catabolism or metabolism, transportation.

Both increases and decreases in a nutrient must be examined for significance as described in Section 10 (Nutrition), while only increases are relevant for Sections 11 (Toxicity) or 12 (Allergenicity).

Gaps in knowledge on pathways and their importance for the nutritional quality/safety of food/feed must be identified, and discussed in Section 13 (Other Safety Concerns).

16.4. Comparators used as references and sources of compositional data

Tier 1 safety assessment relies on the use of comparators and may require provision of compositional data.

Information in this section is specifically required for the FSA food and feed marketing authorisation application, and must be provided as relevant.

16.4.1. Comparators used as references

To assess the significance of any change, suitable comparators must be used to serve as references for the genotype, the phenotype, and the history of safe use of food from the PB organism (HSFU) or existing prior feed consumption (PFC) from the PB organism (see Section 4). When comparators from the same species are not available, a close relative to the species may be an acceptable comparator (for example, wheat, spelt, barley are related species in a same primary gene pool that can inform each other's compositional ranges).

On the existence of the same genomic feature in organisms already available to commercial or home growers:

- Identification of varieties from the same species with a HSFU/PFC: taxonomic information and brief description of the genotype, how it was obtained, how long it has been available in the food chain.

Where the same genomic feature exists in organisms already available in the food chain, it is expected that no additional Tier 2 safety assessment will be required due to the HSFU of the trait.

On any other suitable comparator used in the Tier 1 safety assessment:

- Identification of comparators: taxonomic information including variety and brief description of the phenotype, together with a reasoning for their selection.

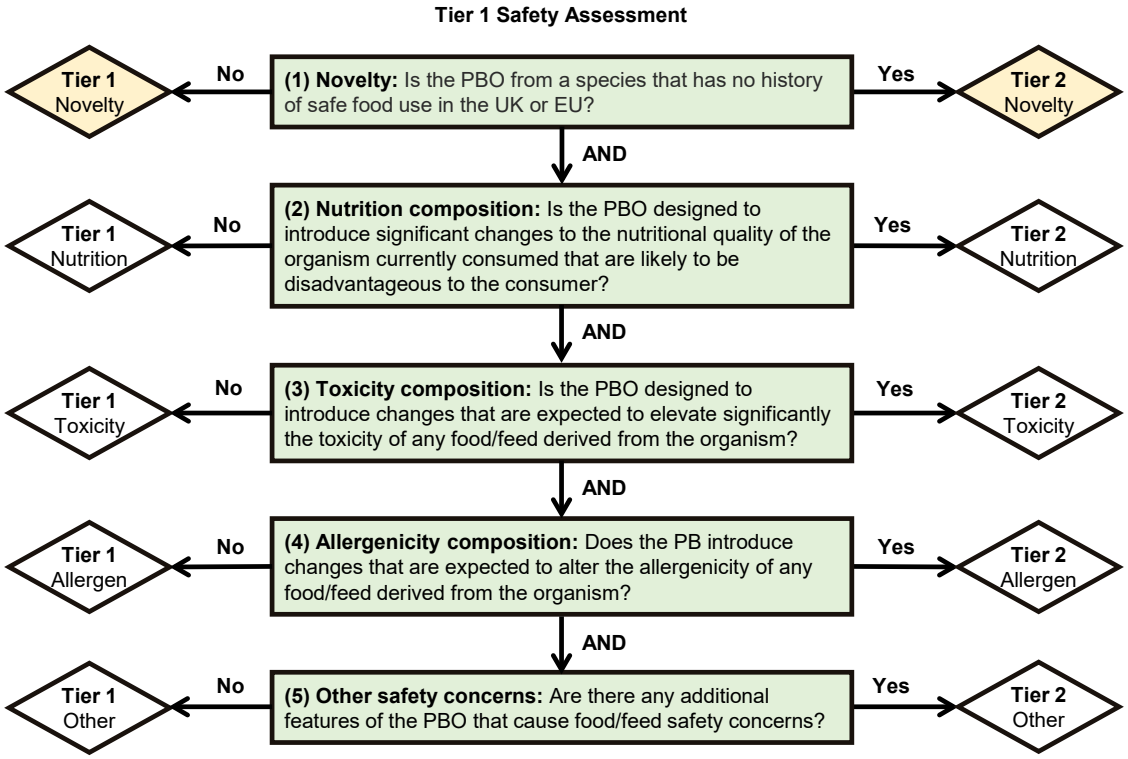
16.4.2. Sources of samples where compositional analysis supports Tier 1 safety assessment

Where applicants draw upon compositional data to support their Tier 1 safety assessment of the PBO, the following must be provided:

- Brief description of the sources of samples; the geographical origin of the crop used to provide the samples for compositional analysis must be specified;
- Scientific reasoning on the criteria for selecting the sampling sources and how they ensure representativeness of crop compositional variability; this must take into account possible secondary effects reasonably anticipated;
- Brief description of the sampling plan, analytical methods and statistical analysis.

Elements to take into account for this are described in Sections 6.6 and 6.7.

17. Information on Novelty to include in all applications



See Section 15 for further instruction on the information to provide in all applications.

The statement on the history of safe food/feed use should relate to the taxonomic species level of the organism (Genus, Species).

Provide:

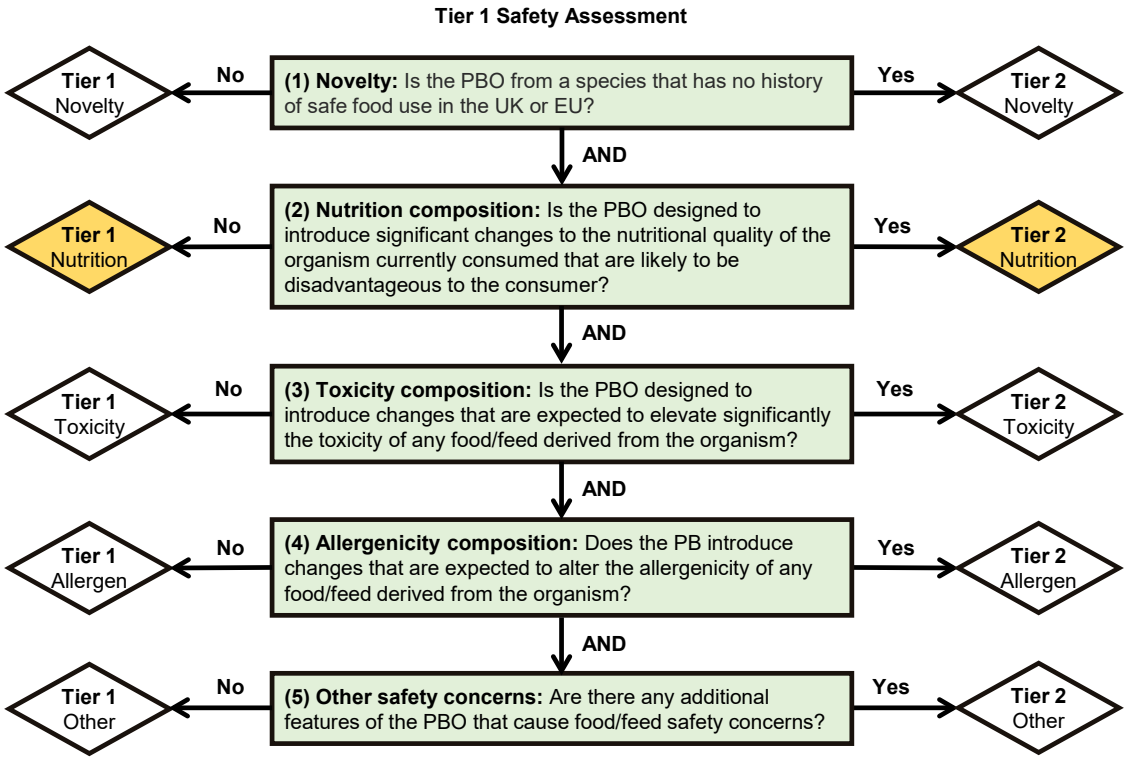
- **A statement concluding on the presence or absence of HSFU**; the history of safe food use within the UK or EU prior to 15 May 1997 must relate to how the PBO is intended to be used as a source of food, note that there might be different histories of consumption for different parts of the organism.

Where it is concluded that there is HSFU of the progenitor organism as food in EU and/or UK:

- Brief description of the extent of the experience of continued use, including details of the population for which the progenitor organism is part of the customary diet, its role(s) in their diet, and the country this applies to.

Applicants are reminded that PBOs which require a Tier 2 safety assessment for Novelty must also be considered for Tier 1 safety assessment of Other Safety Concerns (Section 13).

18. Information on Nutrition to include in all applications



Applicants must use their answers from the Tier 1 safety assessment (Section 10) to identify the relevant information to be provided for Sections 18.1 to 18.6, as described in Section 15. Where compositional data is required, it must be presented as described in Section 5.4.

18.1. State whether the genetic change(s) intentionally alter the quantity of a target nutrient

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The target nutrient that is changed, and whether the change exceeds the normal range found in equivalent TBOs; this must be supported by compositional data as outlined in Section 5.4, provided for each tissue used for food or feed, and demonstrating that the desired phenotypic change has been achieved;
- Whether the nutrient is decreased or increased;
- The results of a literature search of any health risks associated with increased and/or very high levels of the targeted nutrient if the target nutrient is increased;

- Details of any populations that may be adversely affected, if any, along with a short description of methods used including consumption databases used for consumption calculations;
- **For feed**, provide a description of the relevant feed characteristics including the main nutrients provided [for each targeted animals] (i.e source of protein and/or fatty acids and/or calcium and/or carbohydrates etc.)

18.2. State whether the genetic change(s) intentionally alter any antinutrients

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The antinutrient that has been altered, and whether the change exceeds the normal range found in equivalent TBOs;
- Whether the antinutrient content is increased or decreased;
- The results of statistical analyses performed on field trial data quantifying the change in target antinutrient quantity;
- Whether antinutrients are removed by typical processing, together with an identification of the processing step involved in the removal.

18.3. State whether the genetic change(s) alter growing conditions

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The altered growing conditions including likely growing regions, soil conditions, climatic conditions, maturity at which the PBO is harvested;
- The impact of the new growing conditions on nutrient content with reference to the available literature;
- The impact on antinutrient content; if the PBO is intended to be grown in areas with high biotic or abiotic stress, describe the downstream effects of the stress responses with relation to nutrient quality;
- Impact on bioaccessibility including digestibility.

18.4. State whether the genetic change(s) is intended to, or can be reasonably anticipated to alter processing conditions

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The processing step(s) that have been altered, removed or added;

- Any effects relevant to bioavailability, including: factors affecting nutrient production and storage; factors affecting digestibility and absorption, such as fibre production, protein quality, fat content, antinutrient content".

18.5. State whether the genetic change(s) is intended to, or can be reasonably anticipated to alter how food or feed products produced from the PBO will be consumed

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The difference in consumption between the PBO and the comparator(s).

Where no difference in bioaccessibility between the PBO and the comparator(s) is expected:

- A statement confirming no difference in bioaccessibility is expected and summarising the evidence sources supporting this conclusion.

Where no population is likely to be adversely affected:

- A statement confirming no population is likely to be adversely affected and summarising the evidence sources supporting this conclusion.

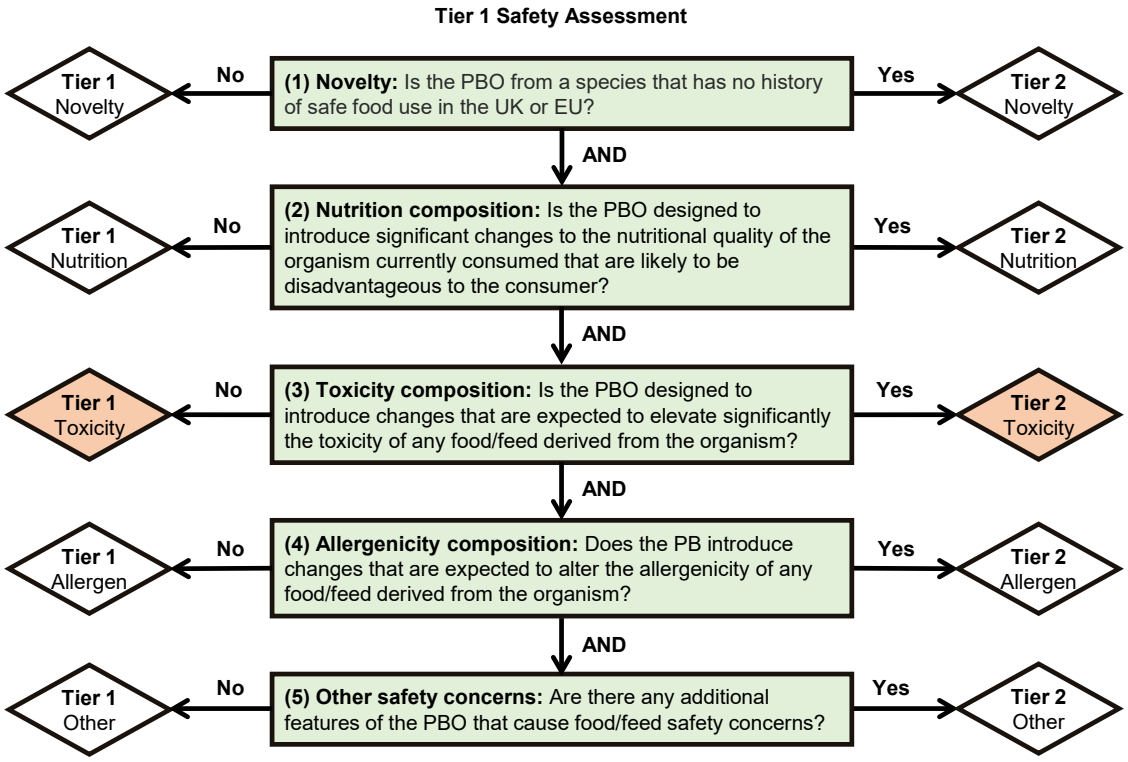
18.6. State whether the genetic change(s) affect the expression of any biological pathway relevant to nutritional quality

If no, briefly describe the evidence sources supporting this conclusion.

If yes, briefly describe:

- The impact on nutrient content and bioaccessibility;
- With reference to the available literature relating to the affected biological pathways, detail any effects relevant to bioavailability including: factors affecting nutrient production, storage; factors affecting digestibility and absorption, such as fibre production, protein quality, fat content, antinutrient content;

19. Information on Toxicity to include in all applications



Applicants must use their answers from the Tier 1 safety assessment (Section 11) to identify the relevant information to be provided for Sections 19.1 to 19.6, as described in Section 15. Where compositional data is required, it must be presented as described in Section 5.4.

19.1. State whether the host plant produces any known naturally occurring or related toxin(s) relevant to food/feed

If no, briefly describe the evidence sources supporting this conclusion.

If yes, provide:

- List of naturally occurring toxin(s) relevant to the safety of food and feed known to the organism, including colloquial and IUPAC names (for non-proteins) or a reference to the database entry in UniProt or GenBank, or similar (where available, for proteins) and brief reference of the evidence sources used to identify them;
- Statement of confirmation that levels of known naturally occurring toxins have been monitored and comply with existing legal limits, or are presumed safe according to HSFU/PFC (within the normal range found in equivalent TBOs or other scientifically reasoned reference).

Where the genetic change(s) **alters the sequence encoding a natural toxin** protein:

- Identification of the target toxin (colloquial and IUPAC names for non-proteins; or a reference to the database entry in UniProt or GenBank, or similar where available, for proteins);
- Description of the structural change: this may use an amino acid sequence alignment of the protein targeted by the genetic change for the PBO and the progenitor, analysed using Protein-families, domains- and signatures-related databases (such as Interpro, Pfam, PROSITE, CATH-GENE3D, SUPFAM, PRINTS, SMART, PANTHER, TIGRFAMS, PIRSF, CDD);
- Scientifically reasoned conclusion on the resulting change in the toxicity of the protein: this may be based on *in silico* prediction methods as reviewed by Palazzolo *et al.* (2020); specify whether the conclusions are based on sequence analysis or published research in peer reviewed journals, the detail of which does not need to be provided in a Regulation 20 application.

Where the genetic change(s) specifically **targets** the production of a naturally occurring toxin (as identified from Section 16.3.3, Description of the genetic change(s)):

- Identification of the target toxin (colloquial and IUPAC names for non-proteins; or a reference to the database entry in UniProt or GenBank, or similar where available, for proteins);
- For each tissue destined for food or feed use: provide compositional data on the targeted toxin as described in Section 5.4. This is both to understand the significance of a phenotypic change relevant to the toxicity, and to demonstrate that the desired phenotypic change has been achieved.

19.2. State whether any natural toxin(s) produced by the cisgene donor are expressed in the PBO as a result of the cisgenesis

If no, where no natural toxins relevant for food or feed are introduced as a result of cisgenesis, justification must be provided, referencing any evidence sources; or confirm where no cisgenesis/intragenesis was used to obtain the PBO.

If yes, where any naturally occurring toxin(s) are expressed in the PBO as a result of the cisgenesis, provide:

- Their identification, including colloquial and IUPAC names (for non-proteins) or a reference to the database entry in UniProt or GenBank, or similar (where available, for proteins), and the mechanism by which their expression in the PBO was made possible: Section 16.3 (Description of the genetic Change(s)) must support this identification;
- Statement of confirmation that levels of the new known naturally occurring toxins have been monitored and comply with existing legal limits, or are presumed safe according to HSFU/PFC (within the normal range found in equivalent TBOs or other scientifically reasoned reference);

- Where specific toxins are further discussed in subsequent steps of the safety assessment: brief review of any health risk associated with known levels of dietary exposure (referenced using body of knowledge from peer reviewed scientific literature); details of any populations that may be adversely affected upon exposure.

Where the **intent** of the cisgenesis is to specifically allow the production of natural toxin(s) new to the organism (as identified from Section 16.3, Description of the genetic Change(s)):

- Identification of the target toxin and brief description of the mechanism of their toxicity;
- For each tissue destined for food or feed use: provide compositional data on the new natural toxin as described in Section 5.4. This is both to understand the significance of a phenotypic change relevant to the toxicity, and to demonstrate that the desired phenotypic change has been achieved in the PBO.

19.3. State whether the natural toxin(s) composition in the consumed tissues is expected to be significantly altered, directly or indirectly

If no, briefly describe the evidence sources supporting this conclusion; this may be inferred from Section 16.3 (Description of the genetic change(s)).

If yes, provide:

- Identification of any natural toxin(s) targeted by the genetic change(s) whose levels are **increased** in PBO compared to the progenitor, according to statistical analysis on compositional data (from Sections 19.1 and 19.2); specify which parts of the plant for food or feed use contain increased levels as a result of expression or transportation (from Section 16.3.3, on the distribution of the phenotype);
- Identification of natural toxin(s) whose toxic activity is anticipated to be **increased** according to functional sequence analysis (from Section 19.1); specify which parts of the plant for food or feed use contain increase levels as a result of expression or transportation (from Section 16.3.3, on the distribution of the phenotype);

Where increases in other naturally occurring toxin(s) can be additionally **reasonably anticipated** as a consequence of the genetic change(s):

- Identification of natural toxin(s) whose levels can additionally be reasonably anticipated to be **increased** according to Sections 16.3.2 and 16.3.4 (Description of the genetic change(s)); specify which parts of the plant for food or feed use may contain increase levels;

- Identification of secondary effects of the genetic change(s) which may be reasonably anticipated to **increase** the levels of known natural toxin(s) in the organism, and of the toxin(s) likely to be affected; specify which parts of the plant for food or feed use may contain increase levels. For example, if the PBO is intended to be grown in areas with high biotic or abiotic stress as a result of the change, describe the downstream effects of the stress responses with relation to the levels of natural toxin(s). Where compositional data have been used to support this application: scientific justification that the sampling plan allows taking into account these secondary effects (see Section 16.4.2).

For each altered naturally occurring toxin increased in the edible tissues of the PBO as identified above:

- Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

19.4. State whether the genetic change(s) is expected to significantly increase the levels of any substance(s) in the consumed tissues, directly or indirectly

If no, briefly describe the evidence sources supporting this conclusion; this may be inferred from Section 16.3 (Description of change).

If yes, provide:

Where the **intention** of the genetic change(s) is to significantly increase the production of any substance:

- Identification of the substance(s) whose levels are intended to be significantly **increased** in the PBO as a result of the genetic change(s); where these are not anticipated to be relevant to the safety of food/feed, provide a brief reasoned justification;
- For each tissue destined for food or feed use, and for each **significantly increased substance identified as relevant to the safety of food/feed**: provide compositional data as described in Section 5.4. This is both to understand the significance of a phenotypic change relevant to the toxicity, and to demonstrate that the desired phenotypic change has been achieved in the PBO.

Where significant increases in other substances identified as relevant for the safety of food/feed can be additionally, **reasonably anticipated** to result from the genetic change(s):

- Identification of substance(s) whose levels can additionally be reasonably anticipated to be significantly **increased** according to Sections 16.3.2 and 16.3.4 (Description of the genetic change(s)); specify which parts of the plant for food or feed use may contain increased levels.

Where significant increases in other substances identified as relevant for the safety of food/feed can be additionally **reasonably anticipated** to result from **secondary effects** of the genetic change(s):

- Identification of secondary effects of the genetic change(s) which may be reasonably anticipated to **increase** the levels of substance(s) in the organism, and of the substance(s) likely to be affected; specify which parts of the plant for food or feed use may contain increased levels. For example, if uptake of nutrients from the environment is increased, describe the downstream effects on the uptake of other substance(s). Where compositional data have been used to support this application: scientific justification that the sampling plan allows taking into account these secondary effects (see Section 16.4.2).

For each substance identified as increased and relevant to the safety of food/feed identified above:

- Statement of confirmation that levels of the substance(s) which could be relevant to the safety of the PBO have been monitored and comply with existing legal limits, or are presumed safe according to HSFU/PFC (within the normal range found in equivalent TBOs or other reasoned reference);
- Scientific rationale to determine significance of the increase, and identification of each substance presenting a significant change: this may refer to typical range found in equivalent TBOs;
- Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

19.5. State whether anticipated processing is expected to remove or reduce the levels of natural toxin(s) / increased substances to or below acceptable levels in food/feed produced from the PBO

In this section, "natural toxins" refer to naturally occurring toxin(s) identified in Section 19.3 and "increased substances" refers to substances identified as increased and relevant to the safety of food/feed in Section 19.4.

If no, briefly describe the evidence sources supporting this conclusion.

Where multiple natural toxins / increased substances are considered in this section, provide the below information for each natural toxins / increased substances that can be successfully managed through processing.

If yes, provide for each natural toxin(s) / increased substance(s):

- Identification of the processing method currently used by anticipated processors as part of food safety management systems to control the levels / activity of the natural toxin(s) / increased substance(s) from the PBO; this must use current knowledge of food safety management systems;

- Evaluation of the efficacy of the methods for removal and/or inhibition using appropriate supporting evidence (references, test results etc); specify whether the conclusions are evidenced by published literature, history of safe processing, or proprietary data;
- Statement of confirmation that levels of the identified natural toxin(s) will be reduced to safe levels through current standard practices of food safety management;
- Where possible, anticipated levels in the food/feed product or range of intended food/feed products must be provided.

Where the trait of the PBO is designed to **improve technological performance of**- or **allow change in**- the **current post-harvest handling and processing** of the organism:

- Identification of processing step(s) that have been altered, removed or added;
- Brief description of whether the change is likely to have implications for the post-harvest management of food safety.

19.6. State whether the dietary exposure to the natural toxin(s) or increased substance(s) could result in adverse consequences for the consumer

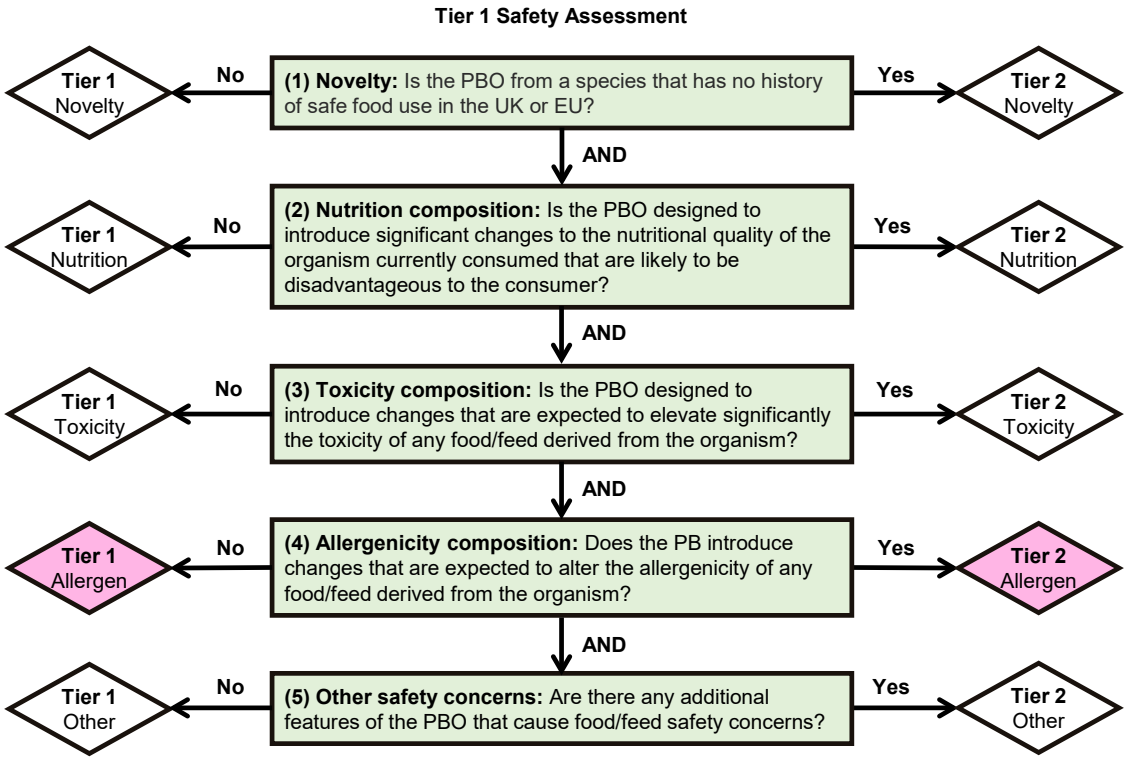
If yes, the additional information to be provided is described in Section 27 (Information to include for Tier 2 FSA safety assessment for Toxicity).

If no, for each natural toxins / increased substances identified in Section 19.5 as not being managed through the food safety management systems of major anticipated processors, briefly describe the evidence sources supporting this conclusion and provide:

- A brief referenced summary of any health risks associated with increased levels of the natural toxins / increased substances, including details of any populations that may be adversely affected;
- The role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups for which the food or feed from the PBO will be a key source of any nutrient; details of consumption databases (such as the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020)) used to conduct the analysis, or the EFSA statement on the animal dietary exposure in the risk assessment of contaminants in feed (2024);
- Brief evidence of HSFU/PFC for UK or EU populations;
- Scientific reasoning for why expected levels of the natural toxins in food/feed as identified in Sections 19.3 and 19.4 are not anticipated to result in significantly increased exposure compared to that expected from equivalent TBOs; specify whether the conclusions are based on predictive or proprietary quantitative information on the levels in the PBO.

Where the **intended use is as part of feed**, the safety assessment must be conducted and evidenced for each different animal consuming the feed, as this may result in different safety concerns.

20. Information on Allergenicity to include in all applications



Applicants must use their answers from the Tier 1 safety assessment (Section 12) to identify the relevant information to be provided for Sections 20.1 to 20.4, as described in Section 15. Where compositional data is required, it must be presented as described in Section 5.4.

The following details **must** be provided:

- State whether the host plant is a clinically relevant allergenic organism
- **If no,** provide a statement of confirmation that the PB species is not an allergenic organism.
- **If yes**, provide a statement that the organism is recognised as potentially allergenic.

20.1. State whether the cisgene donor is a clinically relevant allergenic organism

If no, provide a statement of confirmation that the cisgene donor species is not an allergenic organism.

If yes, state if the allergens are expressed in the PBO as a result of the cisgenesis.

- Description of how it was determined that the transferred genetic material is or is not involved in encoding an allergenic material, for example through using literature searching, or sequence similarity searching in a particular database.

20.2. State whether the allergenic protein composition in the consumed tissues is altered, directly or indirectly

If no, briefly describe the evidence sources supporting this conclusion; this may be inferred from Section 16.3 (Description of the genetic change(s)).

Where the levels of allergenic proteins can be **reasonably anticipated** to be significantly altered as a consequence of the genetic change(s):

- Identification of allergenic proteins whose levels can be reasonably anticipated to be **significantly altered** according to Sections 16.3.2 and 16.3.4 Description of the genetic change(s)); specify which parts of the plant for food or feed use may contain altered levels;
- Identification of the secondary effects of the genetic change(s) which may be reasonably anticipated to **alter** the levels of allergenic proteins in the organism. For example, if the PB is intended to be grown in areas with significantly increased biotic or abiotic stress, describe the downstream effects of the stress responses with relation to the levels of allergenic proteins. Where compositional data has been used to support this application: scientific justification that the sampling plan allows taking into account these secondary effects.

For each altered allergenic protein increased in the edible tissues of the PBO as identified above:

- Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

Where the trait of the PBO is designed to alter the **current post-harvest handling or processing** of the organism:

- Identification of post-harvest handling step(s) that have been altered, removed or added;
- Brief description of whether the change is likely to have implications for the post-harvest management of food safety.

Where the trait of the PBO may allow **new uses from whole, parts or extracts** from organisms:

- Identification of any necessary additional food safety management measures.

20.3. Where allergenicity is expected to be altered, state whether published clinical studies for the same genetic change in this species has demonstrated unchanged allergenicity

If no, briefly describe the evidence sources supporting this conclusion.

If yes, provide:

- A reference to the published study;
- The number of participants;
- The form of the food consumed during the oral challenge;
- Brief summary of the conclusions on allergenic safety;
- Scientifically reasoned conclusion on the safety outcome of the PBO based on it exhibiting the same trait as the variety in the study.

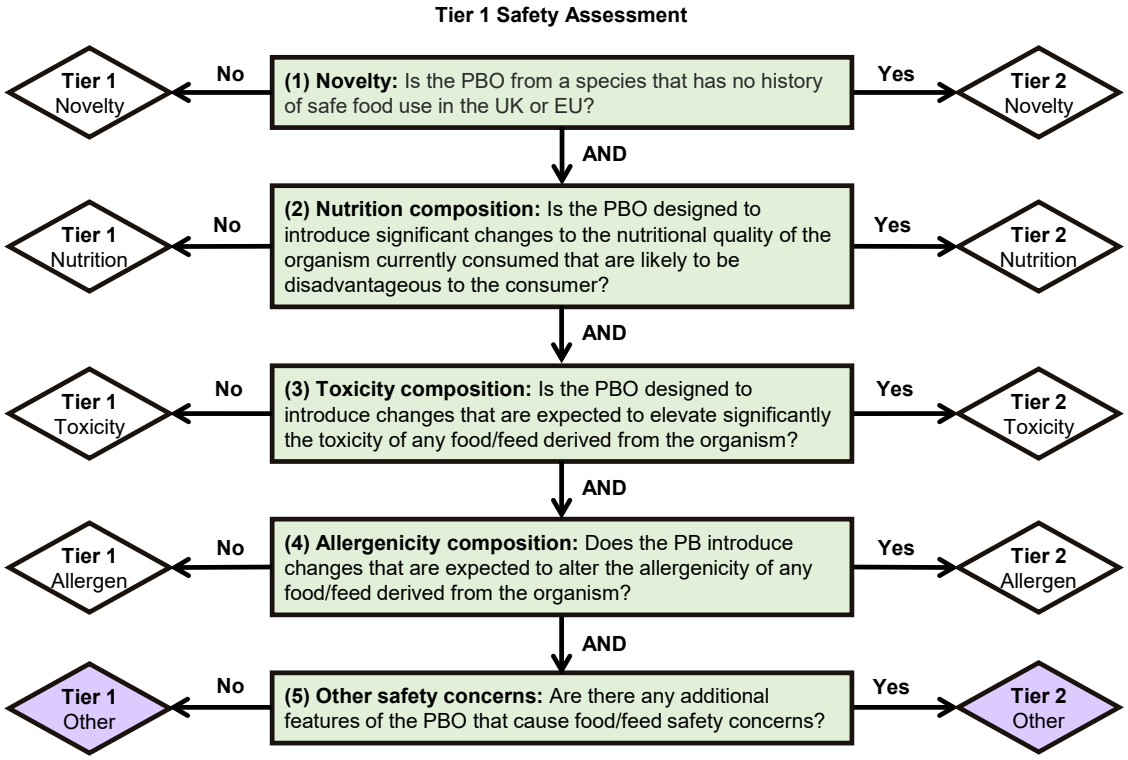
20.4. Where allergenicity is expected to be altered, state whether the same trait in the species, resulting from a comparable genetic change, has a HSFU within the EU/UK

If no, briefly describe the evidence sources supporting this conclusion.

If yes, provide:

- Brief summary of the genomic sequence analysis used to support this section: this must use alignment of the DNA sequence encompassing the genetic change for the PBO and the species with the same trait already on the market;
- Identification of the variety it is compared to, and brief evidence of the HSFU of the trait by EU/UK human populations;
- Scientifically reasoned conclusion on the safety outcome of the PBO based on it exhibiting the same trait resulting from an identical genetic change.

21. Information on Other Safety Concerns to include in all applications



The questions outlined in Sections 13.2.1, 13.2.2, 13.2.3 and 13.2.4 provide a non-exhaustive guide to assessing other safety concerns and give an indication of information to include in a Regulation 22 submission (also see Figure 7). The questions are to be used as a guide only. Applicants must use their knowledge and experience of working with their organism to identify any other safety concerns. It is the applicants responsibility to disclose any safety concerns they are aware of. If any other safety concerns are identified, the PBO requires a Tier 2 FSA safety assessment. For a Regulation 20 application, provide a brief statement confirming no other safety concerns have been identified.

22. Concluding remarks to include in all Regulation 20 applications

The information requested across all the sections must be integrated into a concise overall consideration on how it supports the safety of the PBO under the proposed conditions of use.

23. How to identify additional information requirements for Regulation 22 applications

Applicants must refer to Part 4 to identify the additional information needed for any criterion which requires Tier 2 safety assessment and needs to be provided in an application under Regulation 22.

Part 4 - Additional information to include in Regulation 22 applications to support a Tier 2 FSA safety assessment

24. Regulation 22 applications for Tier 2 FSA safety assessment

Where a Tier 2 safety assessment by the FSA is required for **any** assessment criterion, an application must be made under Regulation 22. These are PBOs where potential food and feed safety risks were identified in one or more of the assessment criteria as set in Regulations 20 (1) (b) and (c). These PBOs will be subject to a tailored case-by-case safety assessment to allow the identified safety concerns to be fully assessed.

Where a Tier 2 safety assessment is required for a criterion, the initial data required always includes the data used in the Tier 1 safety assessment for that criterion, plus a description of the evidence identifying that a Tier 2 safety assessment is required, and any associated data specific to the criterion which requires Tier 2 safety assessment. In addition, any data applicants determine will aid in assurance of safety may be submitted but should be limited to that which is relevant. A PBO can require Tier 2 safety assessment for multiple reasons, even within the same safety assessment question. For each criterion, all safety concerns which were identified during the Tier 1 safety assessment should be described. This enables the FSA to efficiently request appropriate further information to be provided, where necessary, to address concerns identified over the potential for increased risk to consumers.

The additional data required will be case-specific to understand the specific safety concerns that prompted the Tier 2 FSA safety assessment. Therefore, these guidelines are not intended to define explicitly all of the data that might be required in the course of an FSA safety assessment. Genetic alterations that are expected to require an FSA safety assessment are those which cause, or which are expected to cause a non-negligible change in levels of components impacting safety and nutritional quality, including toxicants, allergens, nutrients, anti-nutrients, and other substances that can exhibit non-nutritive physiological effects on humans or animals. This includes changes which are intended to be beneficial to the consumer. These must also be assessed to ensure that altered exposure in the diet will not be detrimental (e.g. over-exposure to normally beneficial nutrients resulting in toxicity). The data for the necessary bespoke assessment may be sourced from that submitted under other regulatory framework guidelines relevant to the issue that prompted Tier 2 safety assessment. The FSA fully supports a reduction of animal testing in risk assessment

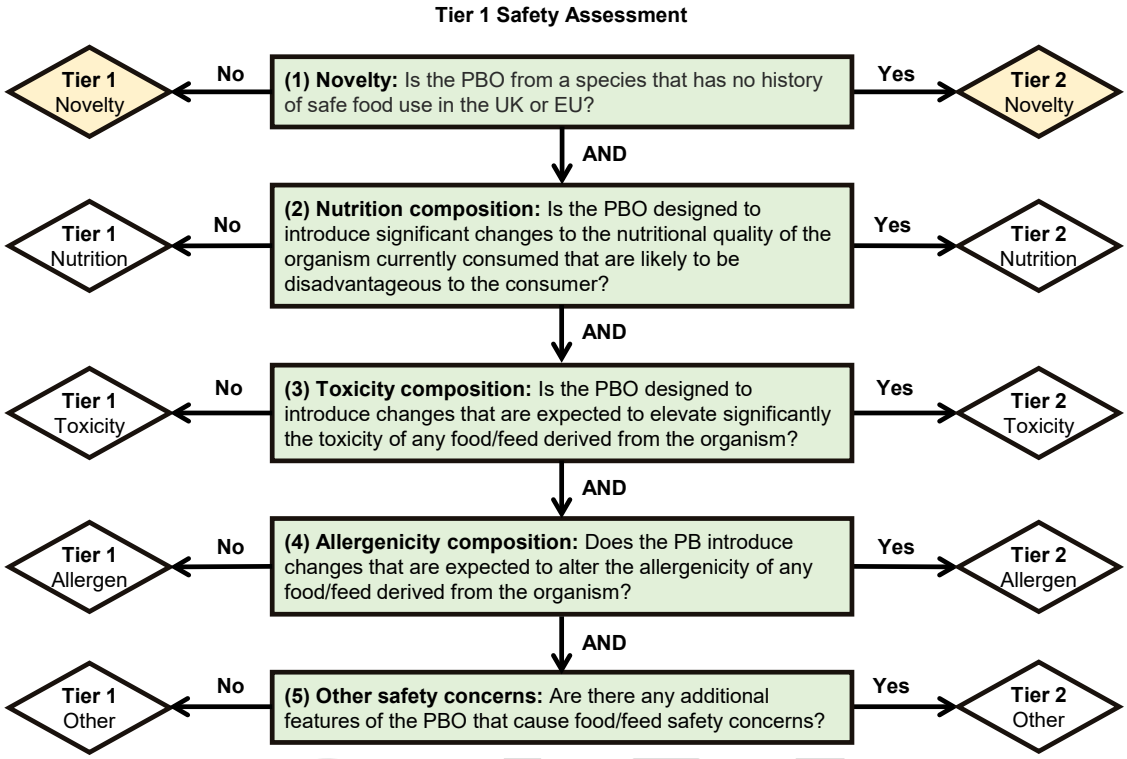
where possible. Further refer to Sections 27.2 and 28.3 of this guidance for details on New Approach Methodologies (NAMs) for the toxicity and allergenicity assessment.

Where key knowledge or methodological gaps are identified, they must be reported. This may prompt FSA safety assessment, unless applicants can make a scientifically justified argument that they do not constitute a safety concern.

Applicants are expected to submit adequate, relevant and concise data. The FSA safety assessment may require provision of sequencing data to support the conclusions.

Following FSA safety assessment, if the safety considerations have been sufficiently addressed, the scientific assessment will provide recommendations for any conditions of use that may need to be managed, if authorised.

25. Information to include for Tier 2 FSA safety assessment of Novelty



See Section 24 for initial requirement for Regulation 22 applications.

When there is experience of continued use of the species **as a source of traditional food** in a third country for at least 25 years from the date of application, this may support the safety of a species as a source of food to be used in its traditional form in the UK or EU. This may mean the safety assessment can be less detailed or in-depth in certain areas. However, the organism must be subject to the necessary assessment to ensure safety of use by the UK population. This is because the UK population will likely have a different overall diet and allergic profile to the country in which the food is regularly consumed. The assessment must also ensure that the trait introduced by PB does not change the organism's safety profile regardless of previous safe use. The information to be provided initially for an application for authorisation under Regulation 22 for PBOs from species with history of safe use for food in a third country (PBOs-OTU) is similar to that requested for a 'Traditional Foods from third countries' application under assimilated Regulation (EU) 2015/2283, but also includes the information identified through Tier 1 safety assessment in Sections 10, 11 and 12.

In contrast, when there is no history of safe use of the progenitor organism **as a source of food** in the EU or the UK prior to 1997 or for at least 25 years in a third country, the PBO from a novel organism (PBOs-NvO) for food must be subject to the necessary assessment based on that for Novel Foods. Where applicants seek an authorisation of

a PBO-OTU not limited to its traditional food uses, they should provide the information required for a PBO-NvO.

The information described in Sections 25.1 to 25.12 **must** be provided unless applicants can justify it is not relevant.

25.1. Identity of the PBO requiring Tier 2 safety assessment for Novelty

In accordance with section 1.3 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c) for both PBOs-OTU and PBOs-NvO:

- The geographical origin of the PBO crop (continent, country, region). Understanding the geographical origin of a crop is important due to the influence of the environmental conditions on the compositional profile of a crop, as described in Section 5.3.

25.2. Compositional data on the PBO requiring Tier 2 safety assessment for Novelty

Compositional data must relate to each part of the organism destined for food use. Analysis must be performed on at least 5 independently produced representative batches of the PBO; this should be performed by accredited laboratories and certificates of analyses provided (see Sections 4, 5)

In accordance with section 3.3 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c), for both PBOs-OTU and PBOs-NvO:

- Qualitative and quantitative characterisation of the main constituents (for example, proximate analyses, i.e. ash, moisture, protein, fat, carbohydrates; mass balance should be calculated; the amount of unidentified components and their percentage relating to the total mass should be indicated and should be as low as possible);
- Comprehensive qualitative and quantitative analysis of naturally derived components which characterise the nature of the organism (for example, peptides, phospholipids, carotenoids, phenolics, sterols);
- Qualitative and quantitative data on nutritionally relevant inherent constituents (for example, micronutrients);
- Qualitative and quantitative data on inherent substances of possible concern to human health (for example, toxic, antinutritive, addictive, psychotropic, allergenic); levels at which the substances of concern derived from the novel organism are present in the respective parts for food must be given where available. The EFSA Compendium of Botanicals (2012) and the EFSA Chemical Hazard Database (2017) may support the identification of such substances;
- Conclusions of a literature search on published compositional data for the organism and the parts used in traditional food.

Provide information on the identity and quantity of residues and chemical and microbiological contaminants (for example, heavy metals, mycotoxins, PCBs/dioxins, pesticides, microbial hygiene indicators and pathogens) relevant to the plant and its production process.

Provide information on the normal storage conditions of the PBO, and identify where stability may be affected as a result of the trait developed through precision breeding (for example, oxidation rate, survival and/or multiplication of contaminating microorganisms).

25.3. Specification of the PBO requiring Tier 2 safety assessment for Novelty

Specification, if necessary, will be generated at the end of the assessment as part of the recommendations for conditions of use.

25.4. Production process for the PBO requiring Tier 2 safety assessment for Novelty

This should identify hazards present in the crop and how these are managed through food-safety management systems used by anticipated processors, in accordance with section 2 of the EFSA guidance on traditional foods from third countries (EFSA NDA Panel, 2024b) and with section 2 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c). In particular:

- Information on the handling of the organism (for example, propagation, growth and harvesting conditions);
- Details on the part(s) of the organism anticipated to be used, and whether they are affected by the genetic change(s).

Where the trait of the PBO is designed to improve agronomic quality:

- Information on whether the trait may adversely affect nutrient bioavailability, consumer metabolism or levels of undesirable substances must be provided, together with evidence how such changes are addressed by post-harvest processing.

Where the genetic change(s) is anticipated to change the occurrence of toxins, antinutrients, nutrients or other substances of interest, in accordance with section 2.1.1.2 of the EFSA guidance on botanicals (2009):

- Information on subsequent processes and how the organism is to be converted into a food product (for example, heat treatment, extraction, purification, distillation, squeezing fractionation, purification, concentration, fermentation, or other procedure(s)).

Where the trait of the PB-NvO may allow new uses from whole, parts or extracts from organisms:

- Identification of any necessary additional food safety management measures.

In addition, for PB-OTU only:

- Information on post-harvest handling and processes and how the organism is converted into a food product in third countries (for example, heat treatment, extraction, purification, distillation, squeezing fractionation, purification, concentration, fermentation, or other procedure(s));
- Description of any change from traditional production processes to industrial, large scale, processes and reasoned evaluation of their impact on the composition and safety of products made of the PBO should be discussed.

In addition, for PB-NvO only:

Where the trait of the PB-NvO is designed to improve technological performance of - or may allow change in - the current post-harvest handling and processing of the organism:

- Identification of processing step(s) that could be altered, removed or added;
- Brief description of whether the change is likely to have implications for the post-harvest management of food safety.

Examples of traits allowing changes in post-harvest handling and processing of the organism include:

- A trait which alters physical properties of the PBO and reduces mechanical requirements in processing;

- A trait that allows a PBO which was traditionally consumed cooked to be eaten raw, making a previously used heat-processing step optional.

25.5. Data from experience of continued use of food from the progenitor of the PB-OTU

In accordance with section 5 of the EFSA guidance on traditional foods from third countries (EFSA NDA Panel, 2024b), relevant literature which may include scientific publications, scientific expert opinions, monographs, information from international or national organisations, governmental documentation, figures on cultivation/harvesting, and sales and trade, should be used to reference the following:

- Brief description of the population groups(s) traditionally consuming food made of the progenitor organism;
- Brief description of the role of the progenitor organism in the diet as traditionally used, and its contribution as micro- and macro-nutrient source. This includes providing figures on frequency and context of the use, the type of meal it constitutes (for example main meal, snack, ingredient);

- Brief description of the handling and preparation of the food made of the progenitor organism, including storage and preparation before consumption (for example: mechanical treatment or separation of parts and use of specific parts of the organism; heat treatment; any other type of treatment);
- Brief description of the precautions for the preparation. This should identify and describe any step taken to reduce levels of antinutrients, toxic or allergenic substances or to improve digestibility;
- Brief description of any restrictions in traditional use by sensitive or specific population groups;
- Brief description of existing available human data demonstrating the safety or identifying hazards (for the whole organism or its main constituents) that require management in relation to toxicology, allergenicity, nutrition, microbiology, tolerance and interaction with medical substances. This may use existing human intervention and observational studies, case reports and surveillance reports.

Any other information relevant to the safety of the PB-OTU and resulting from the experience of continued food use of the progenitor organism must be provided.

25.6. History of consumption of the progenitor of the PB-NvO

Significance of the consumption to establish a history of safe food use is further described in the Information and Guidance document on human consumption to a significant degree (2018).

In accordance with section 5 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c):

- Brief description supported by the literature informing the composition, production and the experience from use of products for food or not for food use, including in countries not UK or EU where available; relevant literature may include scientific publications, scientific expert opinions, monographs, information from international or national organisations, governmental documentation, figures on cultivation/harvesting, and sales and trade.

25.7. Proposed conditions of use of the PBO requiring Tier 2 safety assessment for Novelty

A reasoned argument should be presented for the proposed uses and use levels of foods from the PBO. In accordance with section 6 of the EFSA guidance on traditional foods from third countries (EFSA NDA Panel, 2024b) and with section 6 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c):

- Identification of the target population;
- Description of the anticipated uses **based on the traditional use of the progenitor organism (for PBOs-OTU)** or **based on the properties of the**

organism (for PBOs-NvO), and anticipated use levels. Any intent to replace other foods in the diet must be identified;

- Clear identification of the **role of the organism in the diet** of the target population; this should demonstrate that the use will not be nutritionally disadvantageous. Food from the progenitor organism already consumed in the diet in UK (as determined using the Public Health England NDNS dataset (2020)) has to be provided. Where justified, the role in the diet can be estimated using a comparator (a suitable comparator would be a food that can reasonably reflect the anticipated consumption pattern of the novel organism). Information on the contribution of the food to the overall macro- and micronutrient intake of the population would be helpful
- Identification and justification of any **precautions and restrictions of use**; this should take into account the possibility of overconsumption by some population groups and combined anticipated intakes. For PBOs-OTU, this should build on available information on the safety of the progenitor organism from literature and history of use.

How the proposed conditions of use ensure that identified substances of possible concern are not consumed above upper levels (for example as set in EFSA DRV Finder, EFSA Guidance on tolerable upper intake levels for vitamins and essential minerals, and in COT report on safe upper levels for Vitamins and Minerals (EFSA, 2019; EFSA NDA Panel, 2022; Expert Group on Vitamins and Minerals, 2003), or considering existing Health-Based Guidance Values (HBGVs) as part of total intake) should be discussed; combined intake from the PB-OTU and other sources should also be taken into account.

Where the PBO is intended to be used as a source of a substance in the form of an extract:

- Identification of any further uses of the remaining PBO product after separation, including whether it will be used in other food or feed and disposal methods if relevant.

25.8. Absorption, distribution, metabolism and excretion (ADME) of the PB-NvO

Following the EFSA ANS guidance (2012), it is acknowledged that ‘conventional metabolism and toxicokinetic studies may not be feasible for all components in the mixture, but **should be provided for toxicologically relevant constituents**. Toxicologically relevant constituents are generally considered to be the major components and those other components with known or demonstrable biological or toxicological activity, and should be determined on a case-by-case basis with a scientific justification and the rationale for their selection.‘

Testing for ADME should consider the intended use in food/feed: the test sample must be representative of the part of the organism that will be used in the food or feed produced from the PB-NvO, and where the intended use is in the form of an extract with the potential of concentrating some substances this should be taken into consideration.

Where toxicologically relevant constituents are identified in the PB-NvO, ADME should be assessed in a tiered approach:

- Brief description of absorption and breakdown as reported in the literature, and of chemical and physicochemical data;
- Brief description of *in vitro* absorption data and *in vitro* comparative gastrointestinal metabolism data (to establish whether the substance or breakdown products are absorbed from the gastrointestinal tract).

For nutritionally relevant constituents, the first step should be to address bioaccessibility, digestibility and bioavailability as described in Section 25.9.

Negligible absorption may justify not undertaking higher toxicological testing. Where there is evidence that the constituents are absorbed or are accumulating in the body, the FSA reserves the right to request data from both single-dose administration and repeated dose studies *in vivo* according to according to OECD TG 417 (2010).

When available, data on ADME of the progenitor organism in humans should always be provided.

25.9. Nutritional information on the PBO requiring Tier 2 safety assessment for Novelty

For nutrition safety assessment of PB-OTU, follow the instructions in Section 10.

For nutrition safety assessment of PB-NvO, follow the novel food assessment as described below:

Whether foods from the PB-NvO could be nutritionally disadvantageous for consumers under the anticipated conditions of use is essential to the assessment of the nutritional impact of the novel organism in the diet. Conclusions should be based on details in composition relevant to nutrition (Section 25.2), addressing bioaccessibility, digestibility and bioavailability taking into consideration production, storage and processing prior to consumption with particular regards to known antinutrients; this may include literature searches, *in vitro* and/or *in vivo* testing to address the interaction between the novel food and diet/nutrition. Applicants should take into considerations the needs and risks specifics to vulnerable populations where relevant. In accordance with section 9 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c):

- Brief description of whether the consumption of the PB-NvO is anticipated to result in over-exposure to certain nutrients, based on the role of the PBO in

the diet; identification of any populations for which the PBO will be a key source of any nutrient; including details of consumption databases used to conduct the analysis. The data should be compared to relevant health-based guidance values or upper-level uptakes (as available, for example in EFSA DRV Finder (2019) or COT report on safe upper levels for Vitamins and Minerals (2003)) and to the levels of the nutrient in other foods considered as good sources or major sources of the nutrient in order to understand the contribution of the nutrient to the overall diet;

- Brief description of whether the consumption of the PB-NvO may lead to inadequate intakes of essential nutrients, based on the concomitant uptake of antinutrients or the possible replacement of another source of specific nutrients in the diet. OECD consensus documents (OECD, live database) may be used as reference for this;
- Brief description of whether the PB-NvO is likely to be a new source of micronutrients (for example, biofortification); identification of any populations for which the PB-NvO will be a key source of any micronutrient; including details of consumption databases used to conduct the analysis. The data should be compared to relevant health-based guidance values or upper-level uptakes (as available, for example in EFSA DRV Finder (2019)) and to the levels of the micronutrient in other foods considered as good sources or major sources of the micronutrient in order to understand the contribution of the micronutrient to the overall diet. Note that bioavailability data are essential to the assessment of new sources of micronutrients, as described in EFSA Guidance on scientific principles and data requirements for the safety and relative bioavailability assessment of substances proposed as new micronutrient sources (2024a);
- Brief description of whether the PB-NvO is likely to be a new source of protein and to contribute significantly to the average requirements in protein of any population group; note that data on composition and digestibility (such as Digestible Indispensable Amino Acid Score (DIAAS) value) are essential to assess the quality of proteins.

Further refer to Section 26 (Nutrition) of this guidance for the detail of what must be provided for this section.

25.10. Toxicological information on the PBO requiring Tier 2 safety assessment for Novelty

For toxicological safety assessment of PB-OTU, follow the instructions in Section 11.

For toxicological safety assessment of PB-NvO, follow the novel food assessment as described below:

As introduced in Section 25.8, this section should **focus on toxicologically relevant constituents**. Any new testing that may be needed to assess the toxicity of a PBO

should consider the intended use in food/feed: the test sample must be representative of the part of the organism that will be used in the food or feed produced from the PBO, and where the intended use is in the form of an extract with the potential of concentrating some substances this should be taken into consideration. Section 11.2 may support the identification of relevant constituents needing further assessment for toxicity.

All available knowledge should be examined to determine the need for toxicity studies (EFSA guidance on novel foods (EFSA NDA Panel, 2024c), section 8). This includes: the source, production process, identity and composition of the PBO; any **available** ADME information; any **available** toxicological information on the PBO and its comparator, its constituents or its metabolites (these may be from *in silico*, *in vitro* or *in vivo* studies); any **available** information from human studies; any relevant information or safety assessment from non-food uses of its constituents or its metabolites.

FSA fully supports reduction of animal testing in risk assessment where possible. Further refer to Section 27.2 of this guidance for details on New Approach Methodologies (NAMs) for the toxicity assessment.

A tiered approach will be used to maximise the efficiency of the toxicology assessments and minimise the use of animals. In this hierarchy (tiers) of tests, existing information or simple biological methods will be used first, while tests using cells will only be used subsequently as necessary. Commissioning of additional testing on live animals will only be necessary on the request of FSA; animal testing will only be requested when further safety assurances are needed following initial tests and no suitable non-animal alternative methods exist. Therefore, data requirement will be **on a case-by-case basis**.

Applicants must briefly describe and justify their toxicological testing strategy; this includes justifying when toxicological studies are not needed. Where the intended use is as part of feed, species differences should be considered.

Where further safety assurances are needed, FSA may request applicants to provide further conventional studies of toxicity, following OECD comparative protocols as described in the guidance for submission for food additive evaluations (EFSA ANS Panel, 2012). This may include: toxicokinetics (OECD TG 417); genotoxicity (OECD TG471, TG 487, TG 474, TG 488, TG 489, reviewed in EFSA Scientific Opinion (EFSA, 2011)); subchronic, chronic toxicity and carcinogenicity (OECD TG 408 with extended parameters from OECD TG 407, TG 451 and 452, or combined OECD TG 453); reproductive and developmental toxicity (OECD TG 408 (oral toxicity), OECD TG 414, TG 443, TG 426); neurotoxicity testing (OECD TG 424). All OECD protocols can be found in the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects (2021).

25.11. Allergenicity of the PBO requiring Tier 2 safety assessment for Novelty

For allergenicity safety assessment of PB-OTU, follow the instructions in Section 12.

For allergenicity safety assessment of PB-NvO, follow the novel food assessment as described below:

The allergenic potential of the PB-NvO should consider composition, source, production process, experimental and human data, and cross-reactivity data in accordance with section 10 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c); different requirements may apply depending on the organism and the foods that might be made from it:

Where foods from the PB-NvO are not expected to contain any protein in the form they will be consumed (due to their processing):

- Compositional data confirming the absence of proteins, including method of quantification and its detection limits. No allergenicity data are required; this is because food allergens are mostly proteins.

Where the progenitor organism is related to an organism subject to mandatory allergen labelling (as listed in Annex II of the assimilated Regulation 1169/2011 on food information to consumers (2011)):

- Quantitative data on the known allergens from the organism subject to mandatory allergen labelling.

Where the progenitor organism is not related to an organism subject to mandatory allergen labelling, but belongs to a species known to trigger allergic reactions in susceptible individuals (clinically relevant allergenic organisms can be determined using the current literature, for example the Risk Assessment of Food Allergens, Part 1 (FAO & WHO, 2022a); EuroPrevall UK birth cohort (McBride et al., 2012); FSA Patterns and prevalence of adult food allergies (PAFA) (Simpson et al., 2024)):

- Prevalence of the food allergy related to the organism;
- Type and severity of symptoms triggered by the allergenic food;
- Potency of the allergenic food (for example, minimal eliciting doses of total protein in the food triggering allergic reactions in susceptible individuals);
- Identification of known clinically relevant allergenic proteins of the source; detection and quantitative data on the known clinically relevant allergenic proteins in the PB-NvO.

Where the progenitor organism allergenic potential is unknown:

- Comprehensive summary of the literature on the progenitor organism, on closely related organisms, or on specific trait developed in the PB-NvO, including all types of studies (*in silico*, *in vitro*, *in vivo*, human studies on reactivity, cross-reactivity, elicitation dose, sensitization and clinical effects);
- Protein identification, protein characterisation and allergenicity assessment.

When quantifications of proteins are requested, these should be provided together with the methods of analysis, the LOQ of the methods, and the complete protocol for protein quantification, including the extraction procedure.

Further refer to Section 28 of this guidance for the detail of what must be provided for this section.

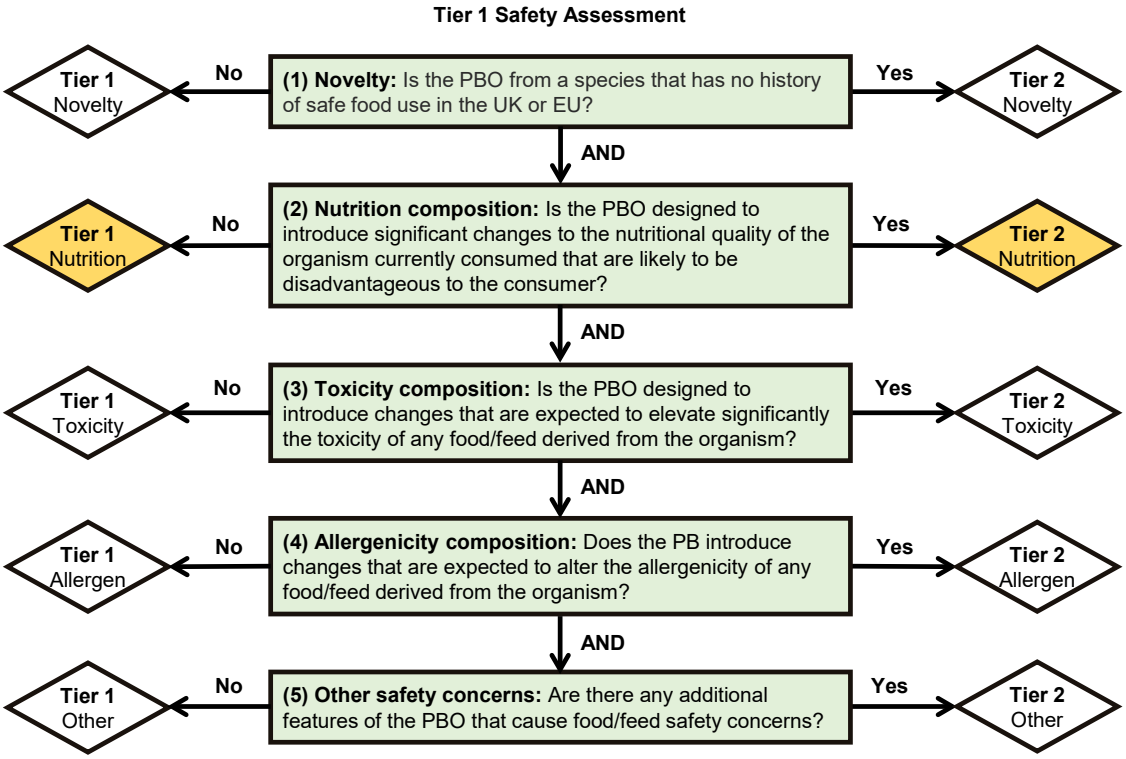
25.12. Concluding remarks on the PBO requiring Tier 2 safety assessment for Novelty

The information requested across all the sections must be integrated in the form of a concise overall consideration on how it supports the safety of the organism under the proposed conditions of use.

For PBOs-OTU, any possible adverse effects identified through composition and experience of use in third countries, and any sources of uncertainty must also be taken into consideration.

For PBOs-NvO, significance of the toxicologically relevant components must be considered in relation to their estimated intakes, possible background exposure, health-based guidance values and results of toxicity studies. Any adverse effects identified through the human data, and any sources of uncertainties must also be taken into consideration.

26. Information to include for Tier 2 FSA safety assessment of Nutrition



See Section 24 for initial requirement for Regulation 22 applications. All nutrition safety concerns which were identified during the Tier 1 safety assessment should be described.

If the analyses and conclusions of the Tier 1 safety assessment indicate there is a likelihood that the introduced change may adversely affect the nutritional quality of the PBO, further safety assessment of nutritional quality will be needed. A Tier 2 safety assessment will consider: digestibility and bioavailability; relevant qualitative/quantitative compositional data; and any other data requirements as may be required. With reference to suitable comparators, applicants must demonstrate that the nutritional quality is not adversely affected. Where appropriate, analysis should be performed in ISO 17025 accredited labs. Provide details of any relevant accreditations, certificates of analysis, GLP certificates.

26.1. Additional data that must be provided for Tier 2 safety assessment of Nutrition

The exact data requirements will depend on the concerns identified during Tier 1 safety assessment. In all cases applicants will submit the raw data used to confirm and characterise the intended phenotype, as well as the testing methods so that the FSA can independently verify applicants' results if necessary. In addition to the

requirements set out below, further data may be requested to complete the assessment.

In addition, the following data is requested if **nutrient content** is of concern:

- **Newly introduced nutrient**: state whether the PBO contains a nutrient that is new to the organism;
- **Proximate analysis:** protein, carbohydrate, fat, vitamin and mineral content;
- **Nutrient -linked phenotypic data:** any phenotypes that may indicate a reduction in food or feed nutritional quality, for example, discolouration, change in size, shape, consistency of parts intended for food or feed use.

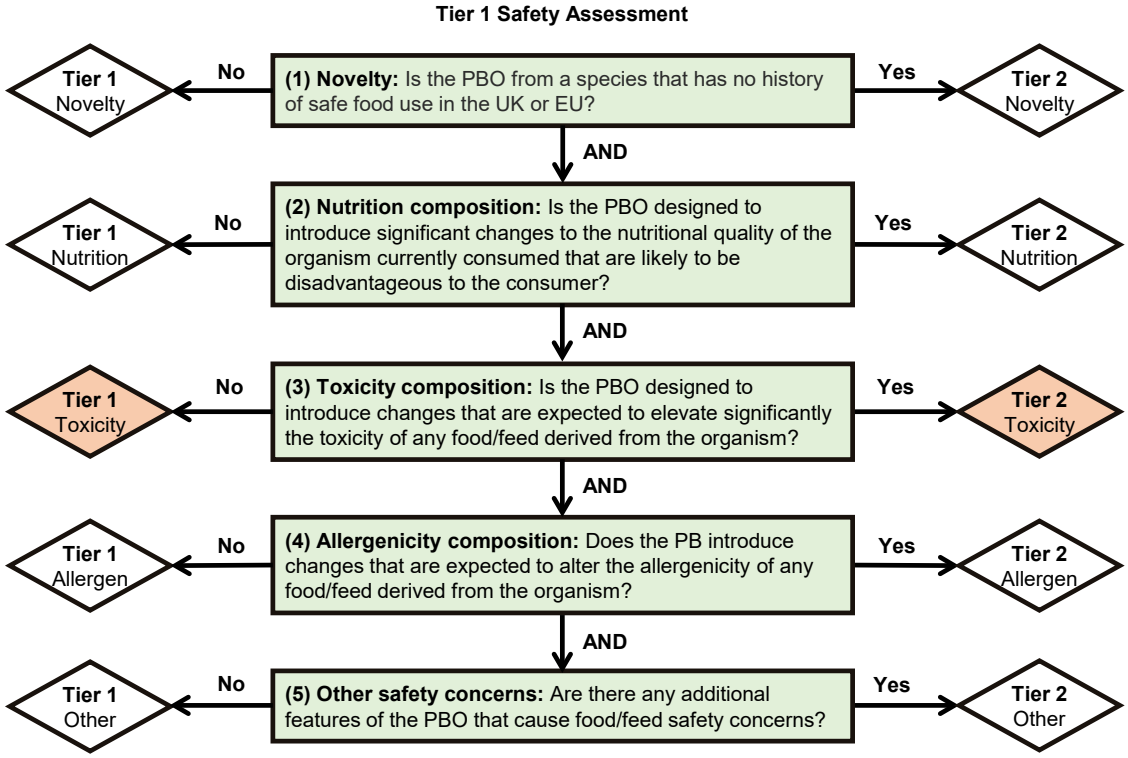
In addition, the following data are requested if **bioavailability** is of concern:

- **Anti Nutritional Hazards:** data relating to any known antinutritional hazards that may be impacted by the genetic change, for example, lectins, oxalates, goitrogens, phytoestrogens, phytates, and tannins;
- **Digestibility Studies –** for example, pepsin resistance studies, proteolytic enzyme studies.

In addition, the following data are requested if **consumption** is of concern:

- **Affected populations**: description of any adversely affected populations;
- **Consumption analysis:** details of consumption analysis as performed in Section 10.

27. Information to include for Tier 2 FSA safety assessment of Toxicity



See Section 24 for initial requirement for Regulation 22 applications. All toxicity safety concerns which were identified during the Tier 1 safety assessment should be described.

This section should **focus on toxicologically relevant constituents**. Testing for the toxicity of a PBO should consider the intended use in food/feed: the test sample must be representative of the part of the organism that will be used in the food or feed produced from the PBO, and where the intended use is in the form of an extract with the potential of concentrating some substances, this should be taken into consideration.

27.1. Additional data that must be provided for Tier 2 safety assessment of Toxicity

The primary set of data required for Tier 2 safety assessment is quantitative data for the **substance(s)/protein(s) which raised concern over toxicity during Tier 1 safety assessment**.

Compositional data must relate to each part of the organism destined for food use. Analyses must be performed on at least 5 representative batches of the PBO independently harvested (as described in Section 5); this should be performed by accredited laboratories and certificates of analyses provided.

Provide:

- Qualitative and quantitative data on the levels of substance(s)/protein(s) of possible concern to human health identified in Section 11.2. Data must include the raw data, the mean, range, and error of the levels of the substance(s). Data must be obtained from each tissue of the PBO relevant for food/feed.
- Comparative analysis with the levels of these substance(s)/protein(s) in already consumed organisms for food/feed with HSFU/PFC.

Where levels of the substance(s)/protein(s) are within the same range as **in other varieties/species** with a HSFU/PFC in the diet, this may be a sufficient assurance of safety.

27.2. New Approach Methodologies (NAMs)

FSA fully supports reduction of animal testing in risk assessment where possible. Where *in silico* or *in vitro* new approach methodologies (NAMs) exist, these will be preferentially used to understand toxicity of a food/feed. When using NAMs as evidence, applicants must describe the validity and biological relevance of their analysis.

NAMs may include bioinformatic analysis, *in vitro*-based cells studies, *in vitro* intestinal digestion studies, supported by a HSFU/PFC (i.e. available information on previous human consumption or on target animal consumption) together with existing previous safety assessments. Further information on the validation of NAMs can be found in the COT FSA UK NAMs Roadmap, expected to be finalised in 2025.

27.3. Experimental design, template and comparator for toxicity assessment

All available knowledge should be examined to determine the need for further toxicity studies (see Section 25.10). This includes: the source, production process, identity and composition of the PBO; any **available** ADME information; any **available** toxicological information on the PBO and its comparator, its constituents or its metabolites (these may be from *in silico*, *in vitro* or *in vivo* studies); any **available** information from human or target animal studies; any relevant information or safety assessment from non-food uses of its constituents or its metabolites.

A tiered approach will be used to maximise the efficiency of the toxicology assessments and minimise the use of animals. In this hierarchy (tiers) of tests, existing information or simple biological methods will be used first, while tests using cells will only be used subsequently as necessary. Commissioning of additional testing on live animals will only be necessary on the request of FSA; animal testing will only be requested when further safety assurances are needed following initial tests and no suitable non-animal alternative methods exist. Therefore, data requirement will be **on a case-by-case basis**.

Applicants must briefly describe and justify their toxicological testing strategy; this includes justifying when toxicological studies are not needed. Where the intended use is as part of feed, species differences must be considered.

27.4. Data that may be requested for Tier 2 safety assessment of Toxicity

Where the levels of the substance(s)/proteins of concern are not within the same range as in other varieties/species with a HSFU/PFC in the diet, information on absorption (see Section 25.8) is needed:

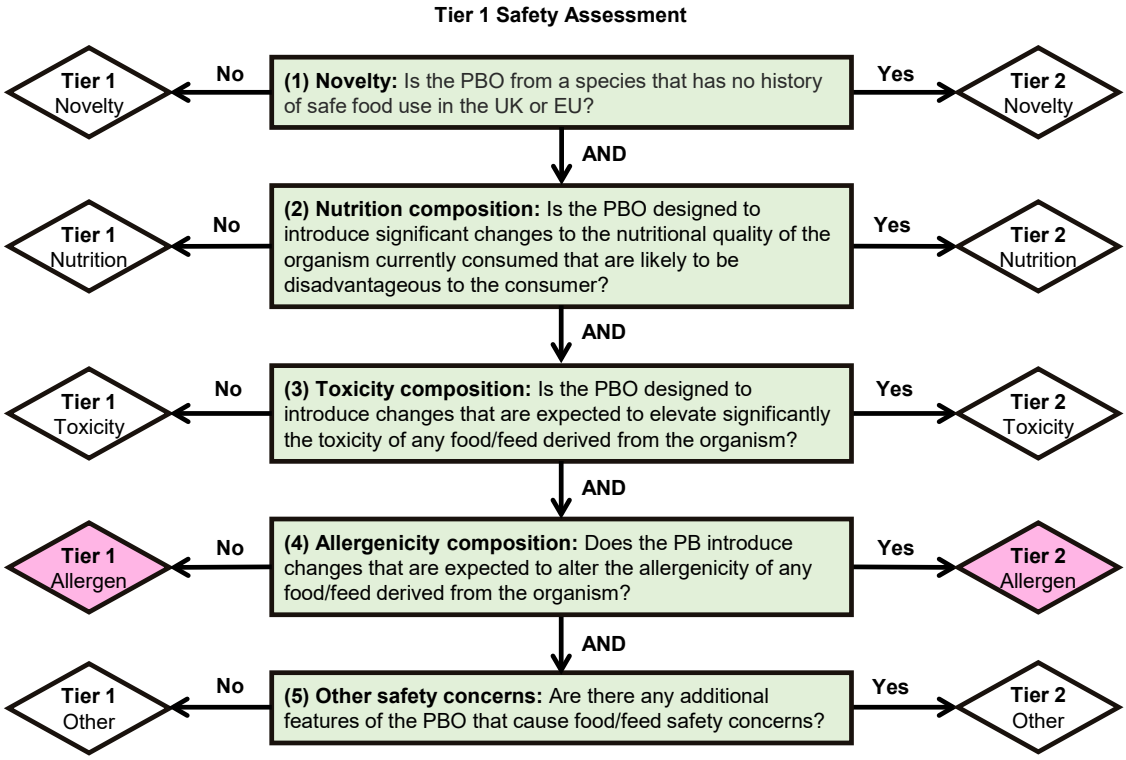
- Brief description of absorption and breakdown as reported in the literature, and of chemical and physicochemical data;
- Brief description of *in vitro* absorption data and *in vitro* comparative gastrointestinal metabolism data (to establish whether the substance or breakdown products are absorbed from the gastrointestinal tract).

Negligible absorption may justify not undertaking further toxicological testing.

Where there is evidence that the constituents are absorbed or are accumulating in the body, the FSA may request applicants to provide data from both single-dose administration and repeated dose studies *in vivo* according to according to OECD TG 417.

Where further safety assurances are needed, FSA may request applicants to provide further conventional studies of toxicity, following OECD comparative protocols as described in the guidance for submission for food additive evaluations (EFSA ANS Panel, 2012). This may include: toxicokinetics (OECD TG 417); genotoxicity (OECD TG471, TG 487, TG 474, TG 488, TG 489, reviewed in EFSA Scientific Opinion (EFSA, 2011)); subchronic, chronic toxicity and carcinogenicity (OECD TG 408 with extended parameters from OECD TG 407, TG 451 and 452, or combined OECD TG 453); reproductive and developmental toxicity (OECD TG 408 (oral toxicity), OECD TG 414, TG 443, TG 426); neurotoxicity testing (OECD TG 424). All OECD protocols can be found in OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects (2021).

28. Information to include for Tier 2 FSA safety assessment of Allergenicity



See Section 24 for initial requirement for Regulation 22 applications. All allergenicity safety concerns which were identified during the Tier 1 safety assessment should be described.

28.1. Additional data that must be provided for Tier 2 safety assessment of Allergenicity

The primary set of data required for a Tier 2 safety assessment is quantitative data for the protein(s) which raised allergenicity concerns during the safety assessment. These should be accompanied by a comparative analysis with the levels of these proteins in already consumed organisms for food/feed with HSFU/PFC, and be provided in the form of a table.

Where levels are within consumed range, including in a different plant species, this might be sufficient to allow a conclusion on safety.

Where the PBO is intended to be allergen-free, the initial data submission must include:

- Identification of the target population.
- Description of the intended use of the final product.
- Description of the final product, including the quantity of the allergen compared to a comparator, and daily intake.

Where the genetic change(s) **alters the sequence encoding an allergenic protein**: Identification of the target allergen;

- Description of the structural change: this should use an amino acid sequence alignment of the protein targeted by the genetic change for the PBO and the progenitor, analysed using Protein-families, domains- and signatures-related databases (such as Interpro, Pfam, PROSITE, CATH-GENE3D, SUPFAM, PRINTS, SMART, PANTHER, TIGRFAMS, PIRSF, CDD);
- Scientifically reasoned conclusion on the resulting change in the allergenicity of the protein; specify whether the conclusions are based on *in silico*, or published research in peer reviewed journals; the detail of which does not need to be provided in Notification.
- For each tissue destined for food or feed use: provide a summary of the compositional data as described in Section 5, and a conclusion on safety/quality. This is both to understand the significance of a phenotypic change relevant to the allergenicity, and to demonstrate that the desired phenotypic change has been achieved in the PBO.

Where the genetic material related to the cisgenic allergen is transferred to the PBO:

- The identification of the allergenic species from which genetic material was transferred, including colloquial name or a reference to the database entry in UniProt or GenBank, or similar (where available), and the mechanism by which the expression of the genetic material in the PBO was made possible: Section 16.3 (Description of the genetic change(s)) should support this identification;
- For each tissue destined for food or feed use: provide compositional data as described in Section 6.7. This is both to understand the significance of a phenotypic change relevant to the allergenicity, and to demonstrate that the desired phenotypic change has been achieved in the PBO.

28.2. Experimental design, template and comparator for allergenicity assessment

This section should **focus on allergenic constituents**. Testing for the allergenicity of a PBO must consider the intended use in food/feed: the test sample must be representative of the part of the organism that will be used in the food or feed derived from the PBO, and where the intended use is in the form of an extract with the potential of concentrating some substances, this must be taken into consideration.

A stepped approach will be used to maximise the efficiency of the allergenicity assessments and minimise the use of animals. In this hierarchy (steps) of tests, existing information or simple biological methods will be used first, while additional tests will only be used subsequently as necessary (only if concern is identified in initial tests). Therefore, data requirement will be **on a case-by-case basis**. Applicants are not expected to submit experimental data (beyond a summary of protein quantification when intentionally changed) unless requested during the Tier 2 FSA

safety assessment. When required, applicants must briefly describe and justify their allergenicity testing strategy; this includes justifying when allergenicity studies are not needed.

Where animal studies are considered to be necessary by the FSA, OECD comparative protocols including number of test doses and control dose, as well as GLP must be followed. For whole food testing, the highest concentration possible of the PBO without causing nutritional imbalance in the laboratory animal diet must be sought.

28.3. New Approach Methodologies (NAMs)

FSA fully supports reduction of animal testing in risk assessment where possible. Where *in silico* or *in vitro* NAMs exist, these will be preferentially used to understand allergenicity of a food/feed. When using NAMs as evidence, applicants must demonstrate the validity and biological relevance of their analysis.

NAMs may include bioinformatic analysis, *in vitro*-based cells studies, *in vitro* digestion studies, supported by a HSFU (i.e. available information on previous human consumption) together with existing previous safety assessments.

Further information on the validation of NAMs for allergenicity assessment as part of a 'weight-of-evidence' allergenicity risk assessment can be found in the EFSA Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology (Mullins et al., 2022).

28.4. Data that may be requested for Tier 2 safety assessment of Allergenicity

A stepped approach to the allergenicity assessment will be requested for a Tier 2 safety assessment where allergenic concerns have been identified. If the requested scientific evidence in the first step does not assure allergenic safety, the FSA may request the next step of assessment is performed until enough evidence has been collected to sufficiently understand safety.

- **Allergenicity-step 1 -** *In silico* bioinformatic analysis to model protein structure or function for allergenicity. Compare the amino acid sequences of the edited proteins with known allergens. Conducted in accordance with the guidelines established in sections 6.1, 6.2 and 6.3 of FAO Allergenicity of Novel Foods;
- **Allergenicity-step 2 -** *In vitro* tests on protein stability and digestibility;
- **Allergenicity-step 3 -** Clinical data: *In vitro* tests (e.g. specific human sera screening studies and/or digestion), skin prick and/or cell activation tests, oral challenge.
    - Clinical oral challenge trials involving appropriate amounts of a derived food ingredient in individuals with well-defined allergies to the source

food of the derived food ingredient remain the gold standard approach to document that the allergenic activity of the derived ingredients is low enough to pose little to no risk to allergic consumers and can therefore be exempted from allergen labelling regulations (Risk assessment of food allergens, FAO and WHO (2022a, 2022b, 2023a, 2023b, 2024)).

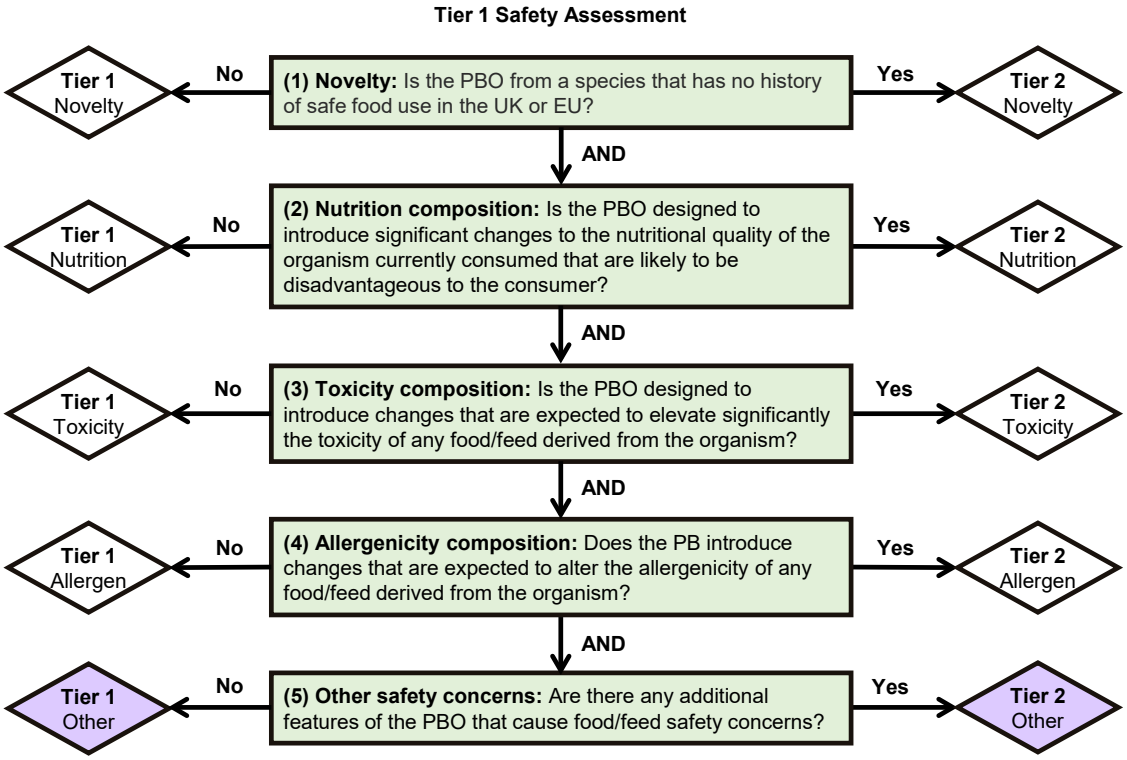
- Evidence of similarity and exposure to the other consumed proteins or species expressing these proteins or similar proteins is needed - reasonable evidence of IgE-mediated oral, respiratory or contact allergy or non-IgE allergy is available on the source of the introduced protein or on the protein itself (Codex Alimentarius, 2009).

Additional information which may be requested includes:

- Demonstration of absence of the allergenic protein in the consumed food/feed;
- Demonstration that the protein quantity is not greater than what is found in TBO comparators;
- Exposure assessment utilising the compositional data of the allergenic protein: detailed description of the role in diet and levels expected in the diet. For example:
  - How does allergenic food contribute to the diet, how does the allergen level compare to other foods with that allergen. Intended use, state what the role is in the diet (replacing a staple or minor component), is there any impact on vulnerable groups (typically children, elderly, pregnant and lactating women).

Demonstration of the absence of biological/clinical reactivity can support a source labelling exemption and may indeed be essential if other data are inconclusive (Risk assessment of food allergens, FAO and WHO (2022a, 2022b, 2023a, 2023b, 2024)).

29. Information to include for Tier 2 FSA safety assessment of Other Safety Concerns



See Section 24 for initial requirement for Regulation 22 applications. All other safety safety concerns which were identified during the Tier 1 safety assessment should be described.

29.1. Other Safety Concerns arising from new conditions of uses

Where the PBO has a new condition of use, provide:

- Clear identification of the new use;
- Recommendation of any new risk management measures if applicable;
- Details of any historic conditions of use associated with the organism;
- For Food use: Description of HSFU;
- For Feed use: Description of PFC.

Where an application is made for Feed use only, provide**:**

- description of any HSFU, and any other relevant information to support the determination of appropriate conditions of use.

29.2. Other Safety Concerns arising from altered processing or storage

Where the genetic change intentionally alters, or could be reasonably expected to alter, processing or storage conditions impacting key food safety measures:

Where the **intention of the change is to alter processing conditions**, provide:

- Detailed description of the process, including a comparison to existing industry methods and an evaluation of the impact on food safety and nutritional quality, including any downstream effects.
- Conclusions should be supported by reference to available scientific literature and compositional data related to the intended change;
- Identification of which storage conditions have been altered. Assessment of the significance of the changes regarding microbiological safety and determine whether an elevated microbiological risk is likely to result.

Where it can be reasonably anticipated that **a processing step will be altered**, provide:

- Evaluation of the impact on food safety and nutritional quality with reference to the food safety management systems of anticipated major processors, and available scientific literature;
- Determination of whether the alterations to processing conditions may impact any microbiological control measures and evaluate whether an elevated microbiological risk is likely to result.

Where a novel process is intended to be used in conjunction with the genetic change to produce an intended compositional or structural trait within a food, the information required is in accordance with section 2.1 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024c); provide:

- Description of the intended trait and the novel process used to obtain it. The description should also include details of the food safety management systems that will be used, identification of any critical control points, safety control checks including verification procedures and associated analytical methods. Provide an evaluation of the impact on food safety and nutritional quality with comparison to the non-treated PBO.

29.3. Other Safety Concerns arising from traits that are new to the species

Where there any changes in the physical morphology that may pose a choking, abrasive, puncture, or other mechanical hazard to the consumer, provide:

- Description of the change in morphology and the way in which the consumer could be harmed, and of any mitigation methods that may be necessary.

Where there are similar combinations of traits in related species that are known to present safety concerns, provide:

- Identification of the relevant traits, description of their known hazards, and of any mitigation methods that may be necessary.

29.4. Other areas of potential safety concern of which the FSA should be made aware

Where there are any gaps in knowledge or methodological uncertainties that hinder accurate Tier 1 safety assessment, provide:

- Identification of what additional information is required.

Where there are any other scientific basis to reasonably suspect the product may present safety concerns, based on the available knowledge of the trait(s), species and mechanism of action, provide:

- Description of any potential safety concerns, and their mechanisms of action.

Additional information may be required on a case-by-case basis as necessary to complete the safety assessment. The exact data requirements will depend on the concerns identified. If applicants have access to further information, for example from internal testing during development, this should be provided for the Tier 2 FSA safety assessment. However, applicants should not commission any new studies until requested by the FSA.

30. Concluding remarks to include in Regulation 22 applications

The information requested across all the sections should be integrated as a concise overall consideration on how it supports the safety of the PBO under the proposed conditions of use.

Acknowledgements

Members of the Advisory Committee on Novel foods and Processes (ACNFP) and its Subcommittee on Products of Genetic Technologies (PGT) who peer-reviewed this guidance as part of ACNFP166 meeting (05/2024).

Abbreviations

Acronym	Definition
ACNFP	Advisory Committee on Novel foods and Processes
ACRE	Advisory Committee on Releases to the Environment
ADME	Absorption, Distribution, Metabolism and Excretion
Defra	Department for Environment, Food and Rural Affairs
DNA	Deoxyribo Nucleic Acid
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organisation
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSA	Food Standards Agency
GE	Genome Editing
GLP	Good Laboratory Practice
HBGVs	Health-Based Guidance Values
HSFU	History of Safe Food Use
IFST	Institute for Food Science and Technology
GM	Genetic Modification
IgE	Immunoglobulin E
NAMs	New Approach Methods
NCBI	National Centre for Biotechnology Information
NDNS	National Diet and Nutrition Survey
OECD	Organisation for Economic Co-operation and Development
PB	Precision Breeding

Acronym	Definition
PB-NvO	Precision Bred from a Novel Organism for food use
PB-OTU	Precision Bred from an Organism with Traditional Use for food
PBO	Precision Bred Organism
PFC	Prior feed consumption
RBD	Refining. Bleaching, Deodorising
RNA	Ribo Nucleic Acid
SAC	Scientific Advisory Committee
SNP	Single Nucleotide Polymorphism
TB	Traditional Breeding
TBO	Traditionally Bred Organism
UK	United Kingdom
URN	Unique Reference Number
WHO	World Health Organisation

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Definitions

Key words	Definitions
Adverse health effects	'Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity to compensate for additional stress or an increase in susceptibility to other influences' (EFSA Scientific Committee, 2017).
Allergen	A protein molecule which leads to an allergic response due to recognition by serum IgE from an allergic individual (Aalberse, 2000), or recognition of gluten proteins due to celiac disease. **Clinically relevant allergen**: An allergen from an organism with a significant severity, potency, and prevalence causing an allergic response in allergic individuals within the UK.
Anticipated Effect	Any effect (desirable or non-desirable) on traits/phenotypes that can be predicted as potentially occurring as a consequence of the intended change. Anticipated effects from the initial submitted data will be considered by the safety assessment process being developed, whereas unanticipated effects cannot be safety assessed unless evidence emerges.
Batch	Group of PBOs of the same species with the same genetic change introduced using the same methodologies so that they express the same phenotype; they typically belong to several cultivars or breeding lines of the same species.
Bioaccessibility	How readily nutrients can be digested and absorbed.
Biological pathway	Sets of steps and activity that contribute to achieve one or multiple related functions in an organism. Biological pathways include regulatory networks, metabolic pathways, and signal(s) transduction pathways.
Cisgenesis	Introduction of a gene, with or without their natural regulatory sequences, and which originate from the genome of a sexually compatible donor.

Key words	Definitions
Comparator	A reference variety with which the PBO is compared.
Composition	The combination of substances produced by the organism that individually and collectively comprise the nutritional, toxicological and allergenic properties of the organism intended for food or feed use.
Direct effect	The immediate phenotypic consequences to the composition of the PBO resulting from the genetic change by precision breeding.
Donor organism	Organism from which an inserted DNA sequence (by cisgenesis or intragenesis) originates.
Feed and feedstuff	Products from plant origin, 'in their natural state, fresh or preserved, and products produced from the industrial processing thereof, and organic or inorganic substances, used singly or in mixtures, whether or not containing additives intended for use in oral animal feeding either directly as such, or after processing, in the preparation of compound feedstuff or as substrates for premixtures'. As set in the assimilated Directive 2002/32/EC on animal feed.
Food safety management system	A set of procedures used by food business operators to prevent consumer illness caused by food hazards.
Genetic change	A specific alteration of the genetic material of an organism. There can be multiple genetic changes introduced by precision breeding in the genome of an organism.
Health-Based Guidance Values (HBGVs)	'Guidance on safe consumption of substances that takes into account current safety data, uncertainties in these data, and the likely duration of consumption' (EFSA, live website).
History of Safe Food Use (HSFU)	A history of safe food use (HSFU) means that the safety of the species in question has been confirmed with compositional data and from experience of continued food use in the customary diet of a significant number of people **in the UK or EU** beginning before 15 May 1997 (Regulation 20 (2) of the Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available]).

Key words	Definitions
	In the Novel Food assimilated Regulation (EU) 2015/2283, which is relevant to non-PB food, it is made clear that traditional foods from third countries should have been consumed in at least one third country for at least 25 years as part of the customary diet of a significant number of people in order to demonstrate a history of safe food use.
Host organism	Organism in which a genetic change is introduced.
Immunoglobulin E (IgE)	Antibodies produced by the immune system involved in most food allergic responses.
Intragenesis	Introduction of genetic material from a sexually compatible donor organism but where the genetic elements have been recombined in a way not found in the donor organism.
Introgression	The incorporation of the DNA from one species into a closely related species through hybridization, followed by backcrossing. Introgression can also be achieved using biotechnological approaches such as cisgenesis.
In silico	Performed on computer or via computer simulation.
In vitro	Performed outside living organisms in a controlled environment, such as in a test tube.
In vivo	Performed in living organisms, typically animal testing or clinical trials.
Marketing Notice	Information provided to the Defra Secretary of State when seeking a precision bred confirmation, as described in Schedules 2 and 3 of the Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available].
Moonlighting	Moonlighting is a phenomenon by which a gene may encode a different physiological function depending on where in the organism it is expressed.
Novel Food	Foods that do not have a significant history of consumption in the United Kingdom or European Union prior to May 1997, as set in the Novel Food assimilated Regulation (EU) 2015/2283.

Key words	Definitions
"Off-target" (genetic) change	An unintended genetic alteration that occurs at a site other than at the intended genomic locations ('on-target' site). When it can be reasonably attributed to the genetic technology/methodology used, the impact on food nutritional quality/safety of any unintended off-target alteration must be assessed in the same manner as intended alteration.
"On-target" (genetic) change	An unintended genetic alteration that occurs at the targeted genomic location. When it can be reasonably attributed to the genetic technology/methodology used, the impact on food nutritional quality/safety of any unintended on-target alteration must be assessed in the same manner as intended alteration.
Phenotype	A phenotype is the physical or observable expression of a trait.
Precision Bred Organism (PBO)	As set out in the Genetic Technology (Precision Breeding) Act 2023: Briefly, an organism that is the product of modern biotechnology where the genetic change introduced is one that could have resulted from traditional processes.
Prior feed consumption (PFC)	Prior use of a feed as part of the diet of a target animal can inform on the safety of the feed; any materials that have already be used for animal feeds in the UK are listed on the Catalogue of Feed Materials.
Processor	A food business operator involved in the manufacture of food and feed products.
Progenitor	Organism from which the PBO is derived – a PBO is obtained by introducing a genetic change into the genome of its progenitor. A progenitor may be used as a comparator.
Reasonably anticipated	Predicted or inferred based on current scientific knowledge (for example based on what is known about the function of the gene affected and its product) or based on existing proprietary data (for example phenotypic observations).

Key words	Definitions
Regulation 20 application	The application route to be used for a PBO where the criteria in Regulation 20 (1) (a) (b) and (c) of the Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available] have been met.
Regulation 22 application	The application route to be used for a PBO where the criteria in Regulation 22 of the Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available] have been met.
Secondary effect	Compositional changes arising from alterations in how the organism is grown, processed and consumed.
Significant (compositional) change	A compositional change is significant if it is outside the ranges found in traditionally bred comparators that have a history of safe food use or of prior feed consumption in the UK or EU, or outside the ranges found in reference food composition datasets, and is biologically relevant to safety/nutritional quality.
Substance	A substance, broadly, refers to chemical components, nutrients, toxins or toxicants that are elements, compounds, or proteins, and are individual constituent components in a food stuff. A substance can be one single chemical entity or can be composed of multiple components.
Targeted (genetic) change	Genetic alteration that occurs at the targeted genomic site and is the intended product of the methodology used for precision breeding.
Thresholds of Toxicological Concern (TTC)	'A screening tool that provides conservative exposure limits in the absence of sufficient chemical-specific toxicological data. It is a science-based approach for prioritising chemicals with low-level exposures that require more data over those that can be presumed to present no appreciable human health risk' (EFSA, live website).
Tier 1 Applicant safety assessment	The initial safety assessment process performed by applicants to determine if Regulation 20 criteria are met, and whether an application should be made under Regulation 20

Key words	Definitions
	or Regulation 22 of the Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available]. To note, a separate and unrelated tiered hierarchy is also used in the approach to the assessment of toxicity and allergenicity, as part of the Tier 2 safety assessment of PBOs, following international procedures.
Tier 2 FSA safety assessment	An additional safety assessment process performed by the FSA after a Regulation 22 application has been received, where Regulation 20 criteria (Genetic Technology (Precision Breeding) Regulations 2025 [insert reference when available]) have not been met. To note, a separate and unrelated tiered hierarchy is also used in the approach to the assessment of toxicity and allergenicity, as part of the assessment of the Tier 2 safety assessment of PBOs following international procedures.
Traditional Food	Foods that do not have a significant history of consumption in the United Kingdom or European Union but are traditionally consumed in other countries and benefit from an history of safe consumption.
Traditionally Bred Organism (TBO)	Organism (plants -including algae- and animals) created by the application of genetic principles in agriculture and animal husbandry, carrying developed or improved desirable traits, obtained through a wide range of conservative tools or traditional processes as described in the Genetic Technology (Precision Breeding) Act 2023 (including sexual fertilisation, spontaneous mutation, *in vitro* fertilisation, polyploidy induction, embryo rescue (plants), grafting (plants), induced mutagenesis (plants), somatic hybridisation or cell fusion of plant cells of organisms which are capable of exchanging genetic material (plants), artificial insemination (animals), embryo transfer (animals), and recovery and transfer of primordial germ cells (animals)).
Unintended effect	A change that was not the objective of the breeding and was not predicted to occur but has occurred and may have consequences for food safety in addition to the intended effect. Unintended effects are inevitable, and also occur in traditional breeding.

Key words	Definitions
Vulnerable Population	Group of people needing specific consideration when assessing nutritional, allergenic, and toxicological effects. This includes for example, such groups as pregnant women, infants, older people, and people with allergies.