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

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To ensure this technical guidance remains current and supports informed decision-making from applicants, it will be regularly updated to reflect the regulator's experience and the development of technologies, tools and databases. Applicants must ensure they are using the latest version of the technical guidance found on the FSA webpage.

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Purpose

This guidance describes the scientific considerations required to evaluate the safety and nutritional aspects of Precision bred organisms (PBOs) prior to seeking a marketing authorisation of PBOs for use in the production of food and feed. This guidance supports applicants understanding how to embed safety management in the plant breeding process.

The purpose of this guidance, provided in five 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).
- To guide applicants through performing an initial hypothesis-driven safety assessment to determine whether a PBO may present potential safety concerns or could be nutritionally disadvantageous (Figure 2, Part 2 and Part 3). Whether an applicant-led Tier 1 safety assessment is sufficient, or whether any additional Tier 2 safety assessment (completed by the FSA) is required, determines whether to apply under Regulation 20 or Regulation 22 of the Genetic Technology (Precision Breeding) Regulations 2025 respectively (Figure 1).
- To help applicants identify the information required to be submitted to the FSA on their Tier 1 safety assessment in applications for a food or feed marketing authorisation under Regulation 20. This same information is also needed as a baseline for applications under Regulation 22 (Figure 3, Part 2 and Part 4).
- To detail the additional information which must be provided when any Tier 2 FSA safety assessment is required and an application under Regulation 22 is made, outlining further information which may be requested during the FSA safety assessment (Part 5).

This guidance is to be used in conjunction with the Applicant Guidance. References to food/feed regulations and obligations under General Food Law (GFL) are included where they support comprehension. However, this guidance does not constitute a guide to GFL, nor does it replace applicants' existing obligations to comply with GFL 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.

Regulatory background – 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. 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. Tier 2 safety assessment is only required in respect of areas where a Tier 1 safety assessment is not sufficient, and should focus on the specific food/feed safety or quality concern(s) identified during the Tier 1 assessment.

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

Applicant-led Tier 1 safety-assessment – All applicants are to perform a Tier 1 safety assessment following this guidance to identify any potential safety concern(s) associated with their PBO.

A step-by-step process of the tiered safety assessment requirements is outlined in this guidance document. The evidence considered by applicants during a 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 benchmark reference. 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 benchmark references that have a History of Safe Food Use (HSFU), or Prior Feed Consumption (PFC) in the United Kingdom (UK) or European Union (EU), or outside the ranges found in reference food composition datasets. Any non-compositional concerns should be considered under "Other Safety Concerns".

On completion of the Tier 1 safety assessment, **applicants** will determine for each criterion whether a Tier 1 safety assessment is sufficient, or whether they consider that a Tier 2 safety assessment by the FSA is required. The FSA will review the validity of all applications.

Tier 2 FSA safety assessment – The Tier 2 safety assessment focusses on the safety/nutritional quality concerns or uncertainties identified by the applicants during the Tier 1 safety assessment. In some cases, a Tier 1 safety assessment may identify safety concerns under multiple criteria (e.g. toxicology, allergenicity and nutrition).

Additional data may be required 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 Tier 2 safety assessment is also needed 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).

Data provision to FSA – This guidance document provides detail on the information which must be provided to the FSA when Tier 1 safety assessment is sufficient and when additional Tier 2 safety assessment is required. 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 rationale, data and conclusions reached by applicants. A verification process will apply to all Regulation 20 applications submitted. This is detailed in the Applicant Guidance. Where Tier 1 safety assessment is sufficient, 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. Where Tier 2 safety assessment is required, additional evidence and detail need to be provided for FSA safety review. The FSA may also request data that was used by applicants in their Tier 1 safety assessment to support the Tier 2 safety assessment.

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Part 1 – Introduction

1. Considerations for all applications

A "Precision Bred Organism" (PBO) is defined in section 1 of the Genetic Technology (Precision Breeding) Act 2023. A PBO has a feature(s) of its genome resulting from the application of modern biotechnology. Only organisms containing genomic changes equivalent to those which could be produced through traditional breeding (TB) are recognised as PBOs. Therefore, potential safety concerns are expected to be equivalent to those found in organisms obtained through TB. As with any breeding process, anticipated effects on the resultant phenotype of the organism and likelihood of it posing potential safety risks to consumers should be considered. Technologies for the generation of PBOs are new and rapidly evolving, therefore any regulatory 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.

This guidance document should be followed by all applicants seeking a PBO food or feed marketing authorisation to ensure an appropriate Tier 1 safety assessment is completed, to satisfy the requirements of the Genetic Technology (Precision Breeding) Regulations 2025 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 trait and the characteristics reasonably anticipated to result from the genetic change. Similar intended traits may be achieved through different biological mechanisms, resulting in differing potential safety concerns. Therefore, the specific genetic change must also be considered.

As PBOs may be a wide range of crops with a broad range of different introduced traits, the assessment hypotheses and appropriate evidence to use will be bespoke for each PBO.

Applicants are expected to embed safety management into their breeding process, with due consideration for food/feed safety and nutritional quality. In seeking a marketing authorisation for food or feed use, applicants must safety assess their PBO with consideration of nutrition, toxicants, and allergens, in addition to novelty and any other safety concerns which may also lead to adverse health impacts. This guidance supports applicants in completing a Tier 1 safety assessment and determining the appropriate application route as well as the information to submit with an application.

Unless otherwise specified, Regulations and Schedules referred to in this document are Regulations and Schedules in the Genetic Technology (Precision Breeding) Regulations 2025.

2. Scope

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 (Precision breeding register: notices and decisions), applicants must complete the safety assessment process outlined in Figure 1 before making an application to the FSA via the correct application 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 any criterion in Regulation 20 is 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 bespoke 'Tier 2' FSA safety assessment focussing on the potential quality or safety concerns identified during the applicant's Tier 1 safety assessment.

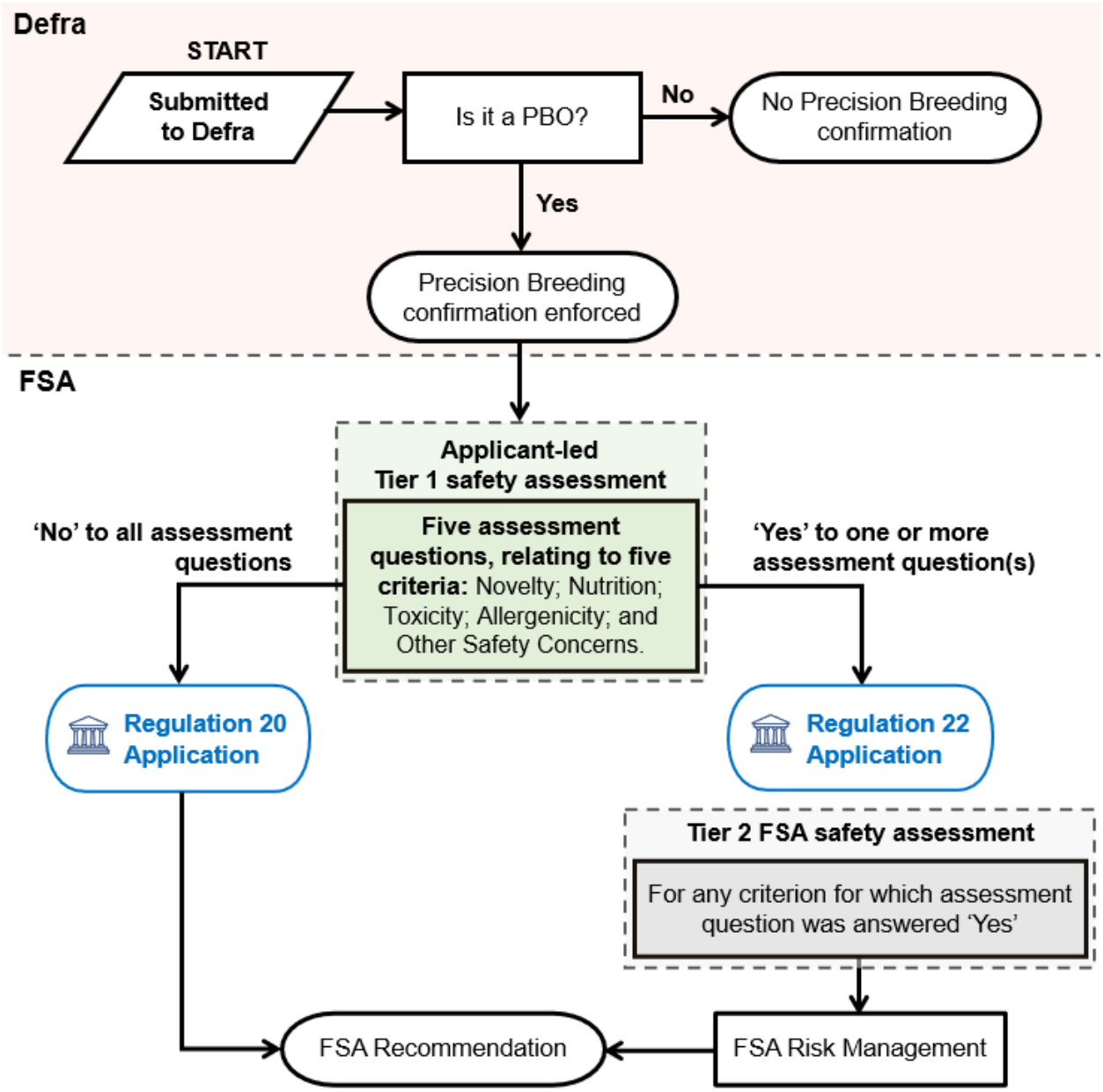


Figure 1.
Overview of the application paths for seeking authorisation of PBOs for food and feed use. Following completion of Tier 1 safety assessment by the applicant, if the answer to all of the safety assessment questions is 'no', PB food or feed marketing authorisation must be sought via a Regulation 20 application. Where the answer to any question is 'yes', or where applicants are uncertain about their answer, an FSA Tier 2 safety assessment is required for the corresponding criterion, and a Regulation 22 application must be made.

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 Part 2). This information must then be used to answer the safety assessment questions (see Part 3, Figure 2).

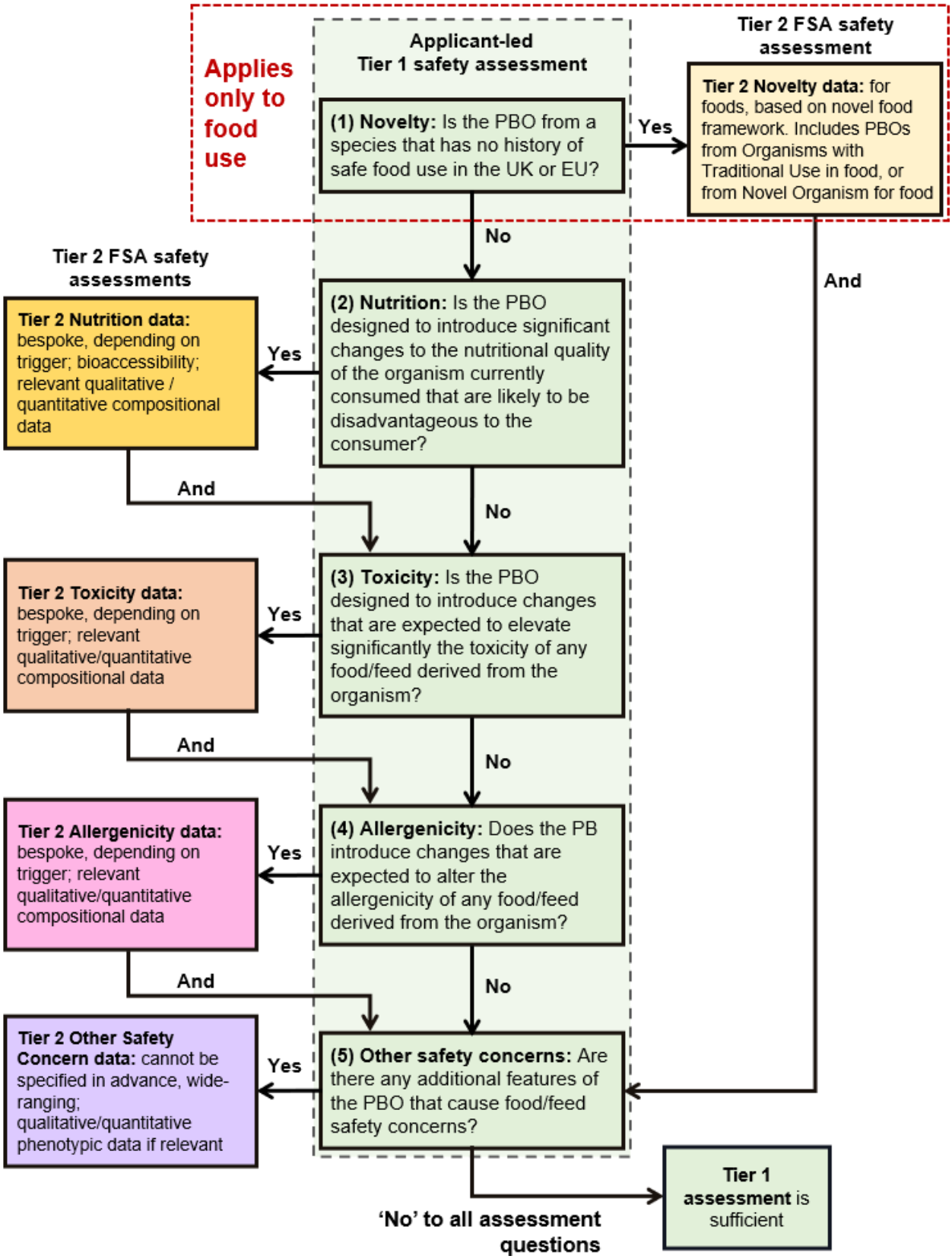


Figure 2.

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 criterion which would trigger the need for a Tier 2 safety assessment, the remaining safety assessment

questions must also be answered. Where the answer to a safety assessment question is 'no', no further safety assessment is needed for the corresponding criterion. Where the answer to a safety assessment question is 'yes', an FSA Tier 2 safety assessment is required for the corresponding criterion. A definition of significance can be found in the table of Definitions.

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 safety assessment for each of these five criteria (Figure 2). The safety assessment questions focus on the immediate phenotypic consequences resulting from the genetic change, taking into account the nature of the genetic change. A phenotype may consist of changes to one or more observable characteristics. Therefore, during the Tier 1 safety assessment, applicants must consider whether genetic changes may be reasonably anticipated to alter levels of substances other than those targeted (including potentially harmful substances), or change nutritional quality (Nutrition). Once the Tier 1 safety assessment is complete, applicants must submit an initial submission including the mandatory information via the appropriate application route detailing their rationale and conclusions (Figure 3).

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, and where applicants cannot sufficiently evidence or are uncertain about the conclusion of 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.

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. **Applicants must provide brief conclusions on the safety of the PBO with respect to each assessment criteria, justified with a summary of the appropriate scientific rationale and evidence utilised.** This should include sufficient details of the characteristics of the PBO to inform businesses and consumers.

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 the rationale and 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.

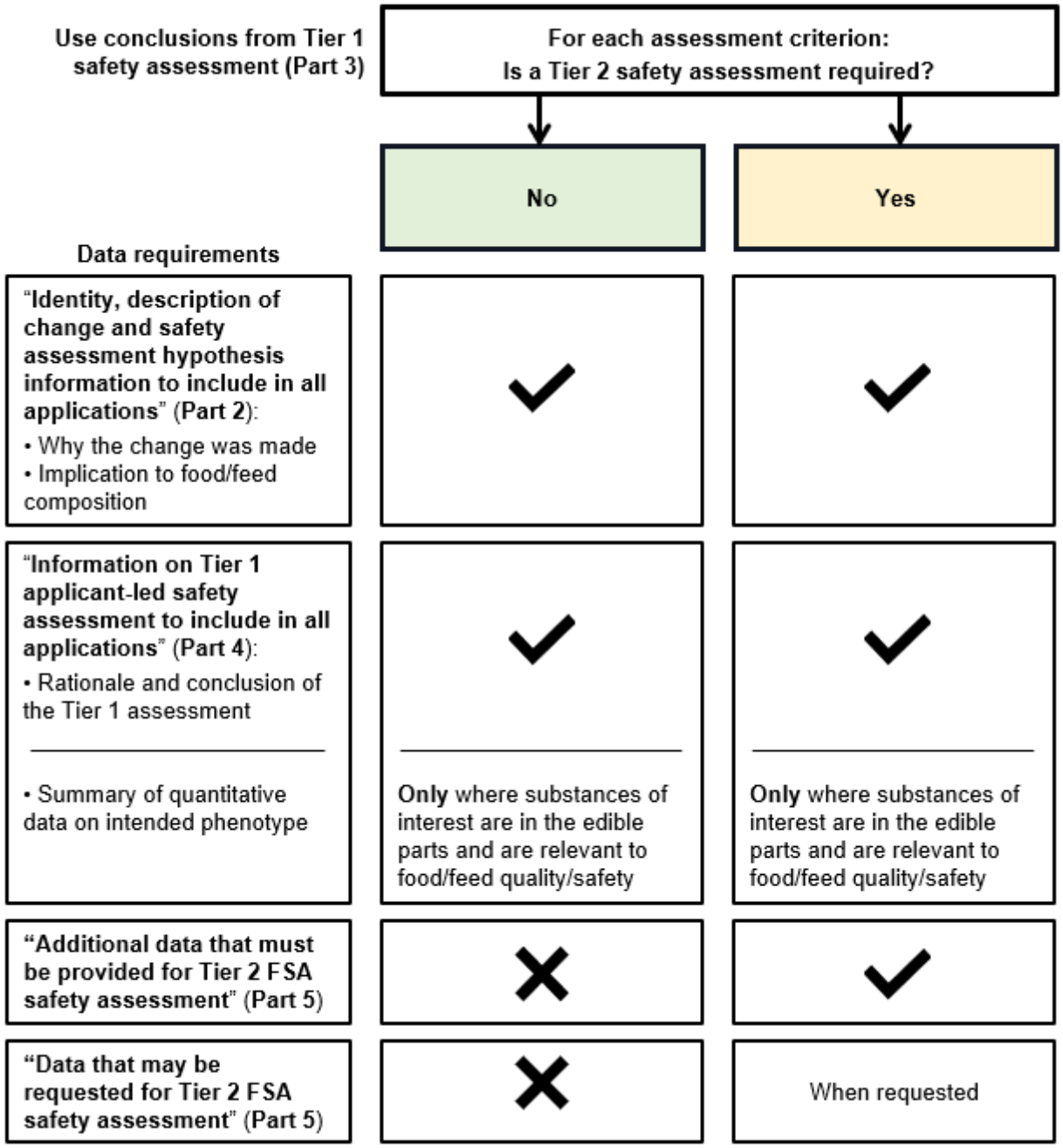


Figure 3.
Flowchart outlining the details of the tiered safety assessment process which apply to each assessment criterion. For each criterion, applicants complete the Tier 1 safety assessment described in Part 3 of the guidance and determine whether a Tier 2 safety assessment is required. Applicants must then submit the appropriate level of data for each criterion to support the required level of safety assessment. A quick reference to the submission requirements can be found in Annex A.

The FSA will verify all Regulation 20 applications as described in the Applicant Guidance 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. Specific considerations

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

- Are novel (have no history of safe food use (HSFU) in the UK or EU prior to 15 May 1997);
- Are submitted as a batch application (see Section 4.2);
- 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.

4.1. Novelty

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.

When a plant has a HSFU, but the trait introduced by PB is designed to allow marketing for food use of a part of it that does not have a history of continued use in the

customary diet of a significant number of people in the UK or the EU beginning before 15th May 1997, 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 15.2.1) to confirm the safety of the use in food of the new part of the plant and identify necessary recommendations for new conditions of use, using information similar to what is required in the EFSA Guidance for Novel Foods (2024c).

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 15.2.2) 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 12.2 (Nutrition), 13.2 (Toxicity) and 14.2 (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 12, 13, 14 and 15.

4.2. Batch applications

A single precision bred Defra Marketing Notice 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).

4.3. 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 15.2.1). 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.

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).

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.

4.4. 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. Prior feed consumption (PFC) may support the safety assessment of feed use.

Similarly, attention must be given to changes in digestibility. Poor digestibility and Bioaccessibility 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.

5. 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 and Part 3 (Applicant-led Tier 1 safety assessment) focuses on the need to understand and explain expected changes in composition and use, to provide assurance that considerations of safety of food and feed have been addressed by applicants. Parts 2 and 3 describe each step of this process with flow charts to determine whether a Tier 1 safety assessment is sufficient or whether further Tier 2 assessment is required for a PBO. This determines whether the 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 4) and what additional information must be included in Regulation 22 applications for those criteria which need further assessment (Part 5).

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 contain 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 information from applicants in accordance with Regulation 24 as part of the verification process.

Part 2 – Identity, description of change and safety assessment hypothesis information to include in all applications

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 taxonomic identity and characteristics pertinent to the identification of the specific PBO must therefore be provided for applications under both Regulation 20 and Regulation 22.

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.

To determine the effects of the phenotype on the safety and quality of food and feed (Figure 4), applicants should consider:

- **Changes in composition which result from anticipated differences to how the PBO is grown, processed, or consumed compared to its traditionally bred counterpart –** This can be inferred from the purpose of the developed trait (Sections 6.4, 6.5), or from how crops with similar trait are grown, processed or consumed. This can be evidenced by breeders' knowledge of the organism and the phenotype, or by peer reviewed scientific literature, industry practice (for example, food safety management systems of intended post-harvest processors, including those of major anticipated processors for Allergenic and Toxicological Hazards), international standards, economic data. Such changes must be taken into account in the assessment for Other Safety Concerns (Section 15).
- **Changes in composition in the phenotype –** The focus of the assessment should be the phenotype and how the genetic change(s) contributes to it; applicants are expected to have data and a good understanding of both. **The assessment should focus on understanding changes that are well established**, either from existing compositional information on plants with the same genetic change giving rise to the same phenotype, or from knowledge of the function and biological effect of the specific genetic change (Sections 6.5, 7). This may also be inferred from current scientific knowledge (for example, supported by rigorous/repeated studies published in the peer reviewed literature). This involves identifying 'substances of interest' (as defined in Section 7). Such changes must be taken into account in a compositional assessment (Sections 12, 13, 14).

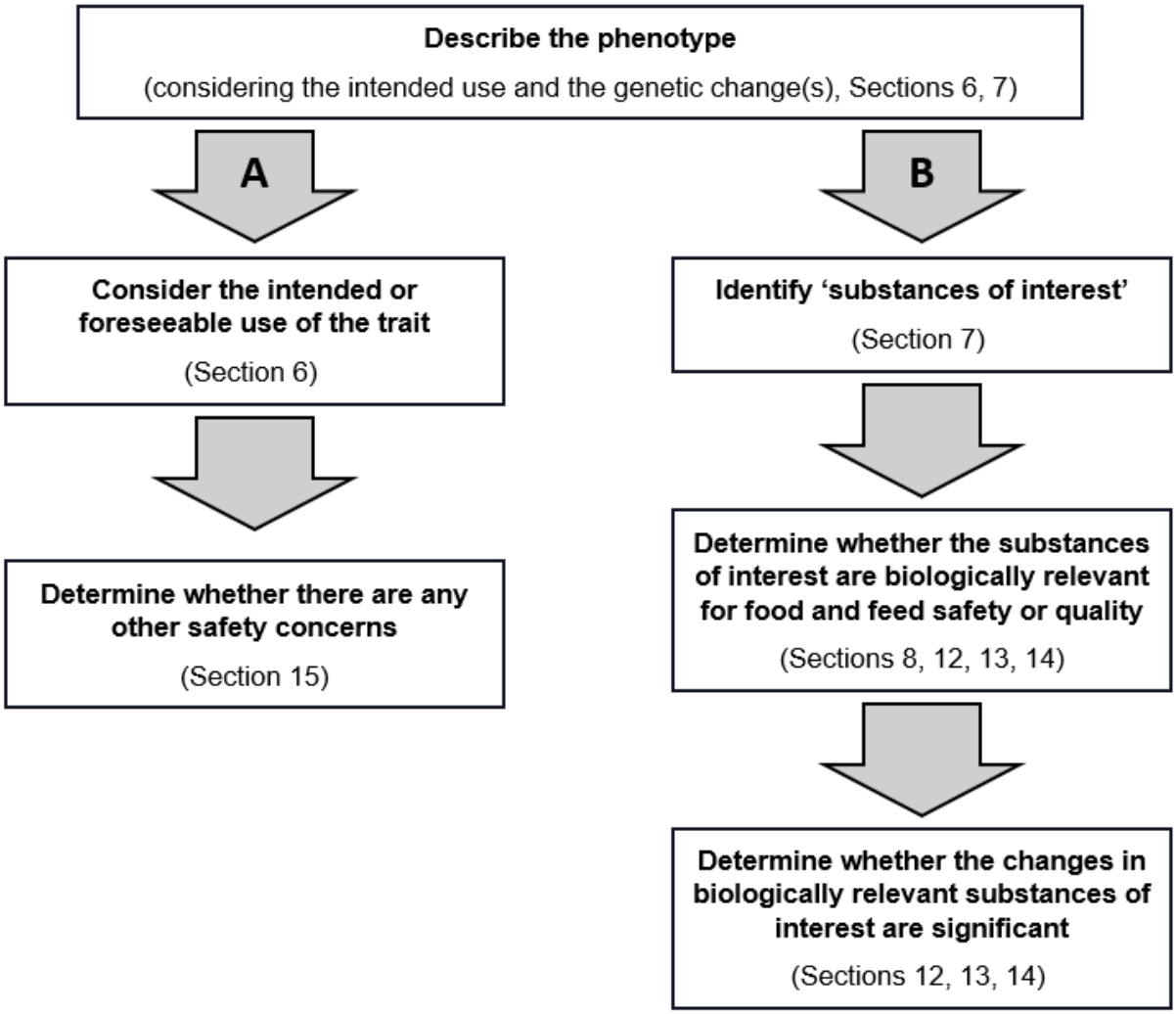


Figure 4

Analysis of the phenotype to generate a risk hypothesis for the compositional Tier 1 safety assessment. Steps in both Path A and Path B must be completed. The applicant first describes the phenotype. **Path A:** From the phenotype, applicants identify intended and foreseeable use of the trait which allows identification of Other Safety Concerns (Section 15). **Path B:** From the phenotype, applicants also identify the 'substances of interest'; these are substances with levels or activity altered as a result of the genetic change, including those changes which can be reasonably anticipated, and which can impact nutrition, toxicology or allergenicity (as defined in Section 7). A step-by-step approach to identify whether the substances of interest are relevant for the quality or safety of food and feed considers: first, whether the substances of interest are in the edible part of the plant (Section 8); next, whether the substances of interest are present at biologically relevant levels (early steps of the assessment described in Sections 12, 13, 14). **Where applicants have sufficient evidence to conclude, using any of these parameters, that a substance is not relevant for the safety or quality of food/feed, the Tier 1 safety assessment is concluded by completing the descriptive statement required in the relevant section(s) of the application form for this substance.** The presence of a novel substance (with no HSFU and PFC) in the edible parts of the plant always requires a Tier 2 safety assessment. Where substances of interest are relevant for the quality or safety of food, the significance of the change must be considered in order to conclude whether a Tier 2 safety assessment is required.

In navigating the safety assessment process, it is necessary to describe the genetic change(s) and understand how the resultant phenotype will compare to that of a traditionally bred counterpart.

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.

6. Information on Identity

Sections 6. 1 to 6. 5 specifically address the requirement in Schedules 4 (1) (3) and (4). Section 6. 5 identifies essential information to evidence conclusions on compositional criteria, in support of the requirement in Schedule 4 (1) (4).

The details listed in Sections 6. 1 to 6. 5 **must** be provided for characterising the identity of the PBO.

6.1. 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.

6.2. 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, the 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 the genetic background may interact with the introduced trait:

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

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.

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.

- Subspecies-specific morphological or physiological features exist, that amplify or diminish the phenotype (for example a variety with no or low epithelial flavonoid will have increased UV exposure of inner cells, impacting any UV-dependant synthesis processes).

- The allergenicity profile of the particular subspecies or variety is different from other subspecies or varieties.

6.3. 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.

  Where there is historical evidence from a similar trait that a trait may provide an opportunity to improve growth/harvest or processing, or allow the PBO to be consumed in different amounts, and/or by a different population, even where this is not the primary purpose, significant impact on composition or diet should be considered (Sections 15.2.2 and 15.2.3, Other Safety Concerns).

  Where the PBO is biofortified, how the change in the nutrient profile will be communicated during marketing must be examined in Section 15.2.1.

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.

6.4. 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).

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

- Whether the PBO is intended to replace another source of food or feed.

Where only specific parts (for example, root, leaf, seed, *etc.*) of the organism are used for **food**:

- The part(s) intended for food use, and whether they are affected by the change introduced by PB.
  Where part(s) of the PBO are new to food use, this must be reported in Other Safety Concerns (see Section 15.2.1).

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 15.2.1).

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.
- Brief description of any intended labelling for the food/feed and/or new condition(s) of use that may support an FSA decision on risk management.

Conditions of use must be reported in Other Safety Concerns (see Section 15.2.1).

6.5. 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):

- 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.

7. Description of the genetic change(s) and identification of substance(s) of interest to generate a safety assessment hypothesis

Tier 1 compositional assessments should be focussed on the phenotype resulting from the introduced genetic change; applicants are expected to have data and a good understanding of both. Applicants should first identify whether the phenotype introduces any changes in composition by identifying 'substances of interest'.

To be 'of interest', substances must fulfil both of the following conditions:

- Their levels or activity must be altered as a result of the genetic change, including those changes which can be reasonably anticipated. Not all changes will be relevant to safety/quality of food/feed. Particular attention should be given to substances that meet the next criterion.
- They have the potential to impact nutrition (substances affecting bioaccessibility, nutrients, antinutrients, adjuvants), toxicity (substances new to food/feed, natural toxins, substances with toxicity by threshold) or allergenicity (allergens).

Where substances of interest are identified, applicants must determine if they are relevant for the quality or safety of food and feed (Sections 8, 12, 13, 14), in which case the significance of the change must be examined (Sections 12, 13, 14). Where changes in composition involve several 'substances of interest', each substance of interest needs to be examined separately.

Where there are no substances of interest identified by the applicant after having taken the steps outlined in Section 7, or where the body of scientific evidence does not provide a hypothesis for a credible impact of a substance of interest on nutrition, toxicity or allergenicity, no further information is required on the substance. The Tier 1 safety assessment in Sections 12, 13 or 14 can be completed by providing a statement outlining the rationale and conclusion for each criterion in the corresponding section of the application form. This does not preclude the need to complete the assessment for other safety concerns described in Section 15.

The following sections describe how to determine whether there are substances of interest in a PBO, which forms the hypothesis from which the composition sections should be navigated.

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 compositional phenotype of the PBO. These represent key considerations and justifications in support of the information detailed in Schedule 4 (1) (5).

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 BLASTN and BLASTX searches) with an available annotated database (for example GenBank, UniProt, String, EMBL-EBI). Homology analysis should be limited to annotated sequences with an experimentally validated function(s) or to sequences with relevant homology to sequences with a validated function(s) (for example functional homologs).
- 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).
- Other sources of evidence can be used where scientifically justified; where an applicant relies upon their own commercially sensitive annotated genome as evidence to demonstrate safety, the genomic data may be requested by FSA, but applicants can request for these to be treated as commercially confidential (Regulation 34).

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. Applicants are not expected to conduct non-hypothesis-based searches or assays to identify new changes. Should evidence emerge following authorisation that alter the conclusions in a way which may affect the safety of food or feed produced from the PBO, it is the responsibility of the applicant to advise the FSA immediately (Regulation 32 (3) (a)).

Characterisation of the change(s) in genomic features at the site of the change or of the insertion of a cisgene/intragene must be briefly described using information obtained during the development stages of the PBO.

The following information is required to perform the safety assessment of PBOs for food/feed use, and **must** be provided as relevant:

7.1. Targeted sequence changes

Information already required as part of the Defra Marketing Notice (Schedule 2 (5) and associated technical guidance):

- 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.
- Where the level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected be change as a result, they are 'substances of interest' which must be clearly identified and be further examined in Section 8.

7.2. Cisgenesis and intragenesis

Information already required as part of the Defra Marketing Notice (Schedule 2 (5) and associated technical guidance):

- 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; and the resulting phenotype which existed in cross-compatible species but were not normally present in the host plant.
- Where reasonably anticipated, clear identification of: gene(s) normally silent in the plant which are now expressed.

- Where reasonably anticipated, clear identification of: gene(s) normally expressed in the plant which are now silent, or which expression is reduced.
- Where the level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result, they are 'substances of interest' which must be clearly identified and be further examined in Section 8.

7.3. 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):

- 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 transcribed region: identification of the specific exon or intron targeted; how this affects the amino acid sequence where relevant.
- Where the genetic change(s) is not in a transcribed region: 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 they are within 1kb of the genetic change; 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: if the insertion may influence the expression of adjacent or overlapping open reading frames or transcripts, direction of the insertion relative to the 5' end to 3' end of the DNA strand (for example based on sequencing).
- 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 compositional phenotype.
- Where the level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result, they are 'substances of interest' which must be clearly identified and be further examined in Section 8.

7.4. Unintended genetic change(s) attributable to the application of modern biotechnologies

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

Where unintended genetic change(s) resulting from the application of modern biotechnology are described in the Defra Marketing Notice (Schedule 2 (6) and (7)) for the PBO, provide:

- Description of the unintended genetic change(s) (as set out in sections 7.1, 7.2, 7.3 and 7.5).
- Where the level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result, they are 'substances of interest' which must be clearly identified and be further examined in Section 8.

7.5. Additional anticipated effects from connection to a biological pathway

Applicants are expected to understand the phenotypic consequences of altering a step in a biological pathway, including transportation mechanisms involved in the movement of substances, according to the current scientific knowledge. Alterations may affect the nutritional quality/safety of food/feed through changes in gene expression and/or the production of more than one related substance. Changes to proteins that are present at very low levels and do not meaningfully contribute to overall protein content or quality are not considered nutritionally relevant, but particular attention should be given to changes in levels or activity of individual proteins that are known toxins or known allergens.

For example:

- A component of a pathway may be a regulatory protein, a target gene, an enzyme, a component of signal transduction, a transporter.

- Interactions may be repression, induction, activation or inactivation, catabolism or metabolism, transportation, and may result from gene regulation, signal disruption, metabolic competition or feedback.

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) with potential to impact nutrition, toxicity or allergenicity 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.
- These are 'substances of interest' which must be clearly identified and be further examined in Section 8.

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

- **Statement**, with rationale as to why no effects on the composition are anticipated.

Where applicants are aware of significant gaps in knowledge on pathways relevant to safety or nutritional quality of food and feed, they must identify and discuss them in Section 15.2.5 (Other Safety Concerns).

Examples of phenotypes with various degrees of compositional changes:

Pod shattering resistance phenotype – Crops with a similar phenotype have no known compositional change and the target gene has no other known well-established function other than that of preventing pod shattering. No substances of interest are identified.

Biofortification phenotype – The nutrient accumulating is part of a well-documented metabolic pathway, and the literature identifies several related substances in the pathway which levels will be increased and decreased as a result of metabolic feedback; each of these substances is a substance of interest.

Stress response phenotype – Modifications made to allow better growth of a potato crop in drought condition. This phenotype is well known to indirectly result in increases in glycoalkaloids levels in the crop. Glycoalkaloids are substances of interest.

Earlier harvest phenotype – The phenotype is achieved by accelerating the maturation well beyond what currently exists for the species to allow earlier harvest; the literature identifies that in the parent of this particular species, vitamins accumulate in the last four weeks of a normal maturation and it is likely that fast maturation will not allow their steady synthesis or accumulation. These vitamins are substances of interest.

8. Distribution of identified substance(s) of interest in the edible parts

A substance of interest 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 identified in Section 6.4.

Information in this section is specifically required for the FSA food and feed marketing authorisation application and must be provided in order to determine the distribution of substance(s) of interest (as identified in Section 7) in the edible parts of the plant intended for food/feed use identified in Section 6.4.

The following information must be provided to determine whether each substance of interest identified is produced in the edible parts used for food/feed:

- **Description of the edible parts of the organism where the genetic change(s) is expected to result in the expression/production of substances of interest**,

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, where well established from the body of knowledge, the following should be considered and reported to determine whether each substance of interest is transported, sequestered or stored in an edible part:

- **Description of known moonlighting of the target gene**, where it is expressed for an alternative function in different edible parts of the organism: this must be informed by available proprietary data and/or peer reviewed scientific literature.
- **Description of transportation mechanisms** which distribute the substances of interest across different edible parts of the organism (including in locations where the targeted gene/function is not expressed), and any resulting compositional changes.

Where substances of interest are identified in edible parts of the plant, the next step is Tier 1 safety assessment of these substances in Sections 12 (Nutrition), 13 (Toxicity), and 14 (Allergenicity).

Part 3 – How to perform an applicant-led Tier 1 safety assessment

9. Sources of evidence to determine the significance of identified changes in substances of interest

Relevant substances of interest identified in Section 8 should be used to form a hypothesis for Sections 12, 13 and 14 of the Tier 1 safety assessment (composition criteria). When required, proprietary quantitative data should be used to establish the significance of the change to food/feed safety and quality by comparison to a benchmark reference. Proprietary quantitative data is only required if both the following criteria are met:

- There is a hypothesis that the substances could have an impact on food/feed safety/quality or use, or the substances have no history of safe food use. This can be identified based on available knowledge (for example peer reviewed scientific literature, databases such as those referenced in Sections 12 (Nutrition), 13 (Toxicity) and 14 (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. Substance(s) that are **not normally found in food or feed** must always be identified and documented with compositional information in Sections 13.2, as relevant, due to the absence of any HSFU or PFC of these substance(s).
- Levels are expected to be biologically relevant in food or feed (for example where only micrograms of a substance are present in the PBO but grams must be ingested to have physiological effects, that substance is not relevant). Levels of substance(s) for which biological **activity** is altered, or of substance(s) that are **new to an organism** commonly consumed, are assumed biologically relevant by default.

Significant changes in composition are those identified as biologically relevant to nutritional quality or to safety, outside the ranges found in comparable traditionally bred organisms from species with a HSFU/PFC in the UK or EU, or outside the ranges found in reference food composition datasets. Significance is used to determine whether a Tier 1 assessment is sufficient or whether a PBO requires further assessment in a Tier 2 FSA safety assessment.

Examples of PBOs with a significant change to a substance of interest may include:

- Those which are known to, or likely to contain substances with no HSFU or PFC in the UK or EU.
- PBOs containing quantities beyond the ranges found in equivalent reference varieties 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 reference varieties such as biofortified PBOs; including changes in pathways related to bioaccumulation such as modifications to biological transporters.

9.1. Benchmark references

To conclude on the significance of the change to any relevant substance of interest, applicants must establish whether a substance of interest is beyond the normal range of what already exists in comparable food or feed. To conduct this comparison they must use an appropriate benchmark reference in order to determine whether the criteria set out in Regulations 20 (1) (c) (i), (ii) and (iii) are met.

Applicants do not need to use more than one relevant reference to conclude on the significance of the change in levels of one substance, but different benchmark references may be used for different substances in the Tier 1 assessment.

Benchmark reference for composition – Scientific rationale must justify the choice of suitable reference varieties, suitable published food composition datasets (for example, McCance and Widdowson, 2021), or suitable peer-reviewed scientific literature. All reference varieties must be selected from non-PBO 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. All compositional data, whether derived from a reference variety, from a published dataset, or from the published literature, must be relevant to the PBO species. When reference varieties from the same species are not available, other species may be acceptable references. For example, close relatives (such as wheat, spelt, barley), or species from a same family (such as legumes, known to share similar nutritional composition) can inform each other's compositional ranges. Any history of use must relate to the same form of use of the PBO, including use of the same edible parts of the organisms, or equivalent role in the diet.

Benchmark reference for trait – Where no compositional information is available for a crop, and the trait is already commonly available on the market in the UK/EU, it may be appropriate to use the trait introduced to the PBO as a benchmark reference. For traits that are new to the PBO, a closely related species (with HSFU and PFC) with the same trait (**resulting from a comparable genetic change**) and with a similar role in the diet can inform conclusions on the safety of the trait, and whether Tier 2 safety assessment is needed for the compositional criteria and "Other Safety Concerns". In

such cases, any history of use must relate to the same form of use of the PBO, including use of the same edible parts of the organisms, or equivalent role in the diet.

HSFU 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 commonly available on the food and feed market.

Benchmark reference for processing – A suitable benchmark reference for processing may include a TBO variety of the same species, which has a HSFU/PFC and shares the same processing properties as the PBO.

9.2. Compositional information on substances of interest and determination of the significance of a change

Compositional information is only required to support the rationale and conclusion of a Tier 1 assessment for identified relevant substances of interest. Data should be provided in the form of a mean value and standard deviation (Figure 5). Should a Tier 2 assessment be needed, the raw data for the relevant compositional information must be provided (Figure 3).

The data used to support the compositional analyses and conclusions in the following compositional sections (12, 13, 14) should reflect commercially-relevant growing conditions. All tests and analyses should be performed competently with suitable quality controls in place, using validated methods, the detail of which do not need providing where Tier 1 assessment is sufficient, but will need providing to support Tier 2 safety assessment where relevant. Applicants should be able to demonstrate (if required) that their results are as robust as those obtained using relevant standards such as ISO 9001 (2015), ISO 17025 (2021), OECD guidelines on Good Laboratory Practice, and Chemical Testing, accredited testing facilities.

Where compositional data is required, it should relate to the parts of the plant destined for food or feed use (Section 6.4), hereafter referred as edible parts. Where the whole plant is expected to contribute to animal feed (for example ensiled plants), applicants must justify how their compositional analysis inform the conclusions on safety/quality of the feed.

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.

The significance of a difference between levels of a substance in the PBO and levels in a suitable benchmark reference can be determined using statistical analysis (for example T-test, Anova).

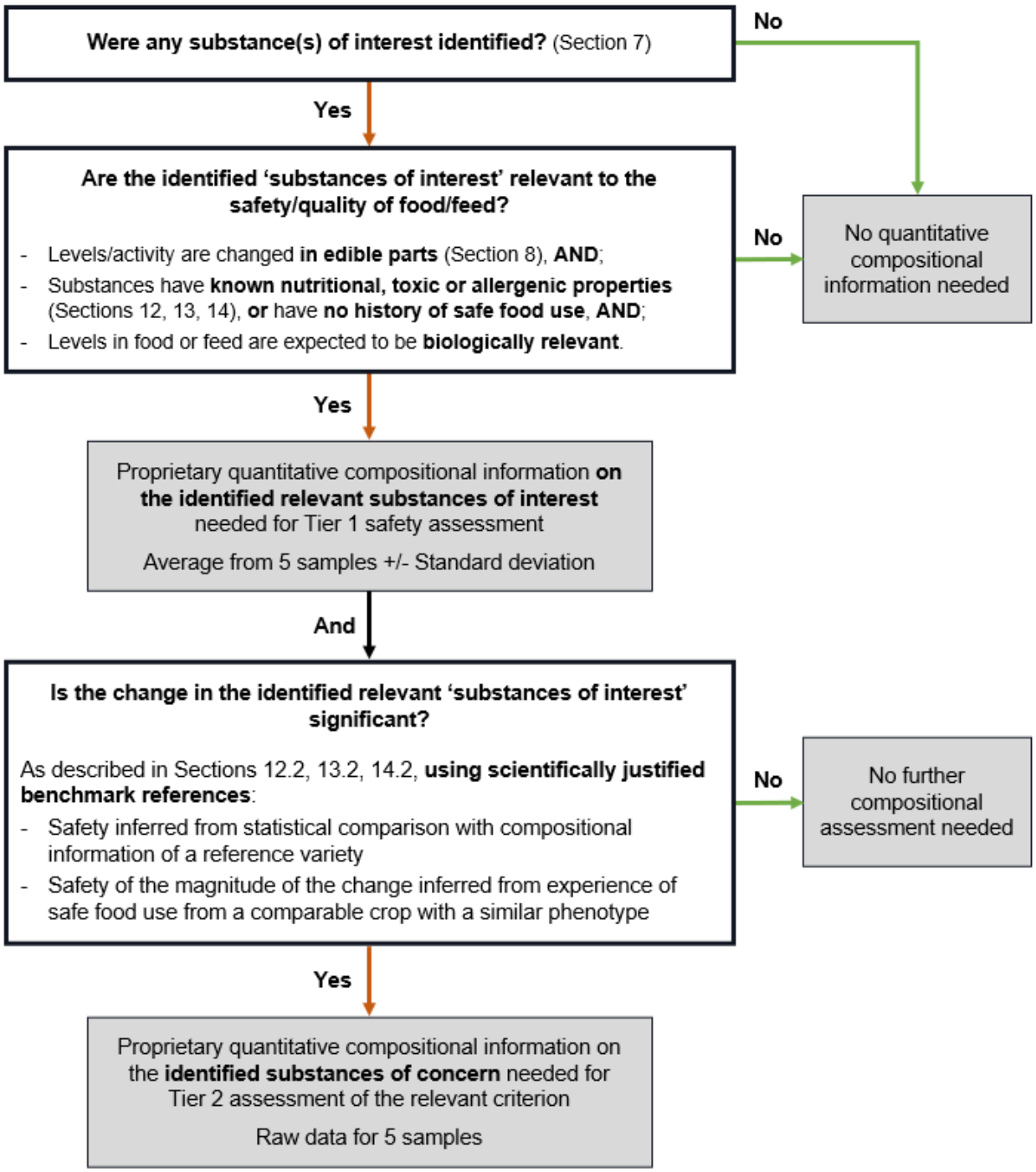


Figure 5.

Compositional information needed for the safety assessment of PBOs. Proprietary compositional data is only required for changes in substances of interest relevant to food/feed safety/quality. A summary of the data is sufficient to document the Tier 1 safety assessment, however raw data are required when a Tier 2 safety assessment is needed.

9.3. Sources of samples, where compositional analysis is required to support safety assessment

Where compositional information is needed to support a Tier 1 or Tier 2 safety assessment, applicants must ensure that the samples are selected using an appropriate strategy. An appropriate sampling plan must have a sound scientific

rationale, reflect real-world growing conditions and possess sufficient statistical power. Any proprietary compositional data held by applicants may be used in support of their assessment, provided they are substantiated by a sufficient number of representative samples and are scientifically relevant.

The detail of the sampling plan does not need to be provided where Tier 1 assessment is sufficient, however applicants should confirm that the samples were obtained from organisms grown using conditions representative of those during food/feed growth (this may be contained growth or field conditions). Further details on the sampling plan may be requested during Tier 2 safety assessment. Particular attention should be given to:

- **Choice of benchmark reference:** see Section 9.1.
- **Endpoints:** Appropriate compositional and phenotypic endpoints must be used for comparative analyses. Particularly, phenotypic data that are linked to allergenicity, toxicity and nutrient quality. This may require samples collected under relevant stress conditions where appropriate for the phenotype. Intended major alterations in growth conditions should be considered.
- **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 from the PBO and from one benchmark reference should be selected for analysis.**

Further information on sampling plans can be found in the Guidance on Good Experimental Practice (sections 3.1-3.4 of the European and Mediterranean Plant Protection Organization (EPPO) Standard PP1/181 (2022)).

10. Tier 1 safety assessment questions

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 4.1). The Tier 1 safety assessment focuses on changes to the following compositional and non-compositional 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) –** Characterising 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 must also be considered.
- **Other safety concerns –** Non-compositional changes that may impact safety, or new uses that may cause an identifiable food safety issue, must be considered in Other Safety Concerns.

Each criterion, described in an individual section in the guidance, must be navigated by completing the sub questions, where 'yes' or 'no' answers will determine whether further assessment is required in Tier 2. **The sub questions represent the 'key considerations' applicants should assess and evidence to demonstrate how they reached their conclusions in relation to the criteria set out in paragraphs (1) (b) and (c) of regulation 20 (which are referenced in Schedule 4 (1) (5))**. Tier 2 assessment is only required for the specific criteria for which the safety assessment question (Figure 2) was answered 'yes'. The rationale and conclusions drawn during Tier 1 safety assessment must be provided in the data submission. A summary of the submission requirements based on the conclusions for Tier 1 safety assessment is provided in Annex A. Annex A can be used in conjunction with the flowcharts summarising the Tier 1 safety assessment for each criterion in Part 3.

For each of the following Tier 1 safety assessment sections:

- 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 nutrition, toxicity or allergenicity expected between the different PBOs within the batch must be clearly identified for each question.
- 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 15.2.1).
- Where the **intended use is as part of feed**, the safety assessment must be conducted for each different animal consuming the feed, as this may result in different safety concerns.

11. Novelty Tier 1 safety assessment

11.1. Introduction to Novelty

This section 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.”

“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 6.

TBOs for food use that have no HSFU are subject to 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. 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 26; the type and amount of information to provide for a Tier 2 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)**.

Feeds that are from species new for use in feed are not subject to Novel Food regulations; feed businesses are to exercise due diligence in considering the safety risks of derived feed products. When PBOs are developed for feed use only or may be used for feed, and are from species with no PFC, their novelty must be taken into account during their Tier 1 safety assessment as described in the compositional and ‘Other Safety Concerns’ sections of this guidance (Sections 12, 13, 14 and 15). The outcome of Tier 1 safety assessment of novelty for the **feed use of PBOs** will never be a requirement of Tier 2 safety assessment for Novelty, and the correct application route will be determined by the responses to the other assessment criteria.

PBOs intended for food use only, for feed use only, or for both food and feed use, require different approaches to the tiered assessment (Table 1)**:**

- 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 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.
- When PBOs are intended for feed use, whether this is in addition to food use or not, applicants must complete the Tier 1 safety assessment of the feed use for Nutrition, Toxicity, Allergenicity and Other Safety Concerns.

Table 1. Required Tier 1 applicant safety assessments for PBOs for food and for feed use depending on whether the PBO is from a species with a history of safe food* use in the EU or UK prior to 1997

	Tier 1 Safety Assessment **Nutrition** (Section 12.2)	Tier 1 Safety Assessment **Toxicity** (Section 13.2)	Tier 1 Safety Assessment **Allergenicity** (Section 14.2)	Tier 1 Safety Assessment **Other Safety Concerns** (Section 15.2)
Where the PBO is from a species **with** a History of Safe Food Use *	Needed for food and for feed use	Needed for food and for feed use	Needed for food and for feed use	Needed for food and for feed use
Where the PBO is from a species **without** History of Safe Food Use *	Not needed for food use # Needed for feed use	Not needed for food use # Needed for feed use	Not needed for food use # Needed for feed use	Needed for food and for feed use

* A "history of safe food use" is 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 food use of PBOs with no history of safe food use safety assessment must be submitted to a Novelty Tier 2 safety assessment, which covers nutrition, toxicity and allergenicity; note that the flowcharts describing Tier 1 safety assessment for these specific criteria may support applicant in identifying what concerns they need to address and what information they need to provide to support Novelty Tier 2 FSA safety assessment.

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

Where a PBO is intended for food use, safety assessment must be completed as described in Section 11.2 (Figure 6). Additional novelty considerations for PBOs intended for food or feed use are described in Section 11.3.

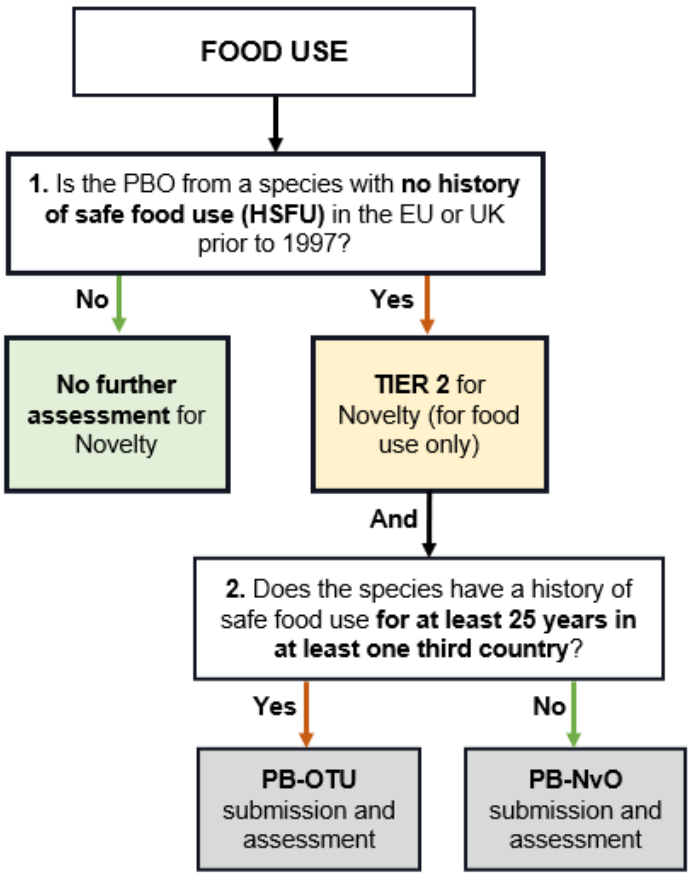


Figure 6

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?**" 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 History of Safe Food Use (HSFU). Different Tier 2 assessment approaches are required for 'PBOs from Organisms with traditional use in food' (**PBs-OTU**) or for 'PBOs from novel organism for food' (**PBs-NvO**). For detailed instructions, refer to Section 11.2.

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 this 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. Relevant sources of information to determine the novelty of a PBO include evidence that crops are commonly grown or used in the EU or UK (seed registers, official agricultural statistics, importation data, EU Novel Food status catalogue).

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 (see Section 11.3).

If the answer is Yes: A Tier 2 safety assessment for Novelty is required; this ends the safety assessment of novelty of the food use of the PBO. Proceed to Step (**2**). Also complete Tier 1 safety assessment in Section 15 (Other Safety Concerns), but not in Sections 12 (Nutrition), 13 (Toxicity) and 14 (Allergenicity).

Step (2) – 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 No: An FSA safety assessment of the PB-NvO, similar to other Novel Foods in the context assimilated Regulation (EU) 2015/2283 information described in Section 25 should be provided.

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. Applicants seeking an authorisation of a PBO-OTU not limited to its traditional food uses should provide the information required for a PBO-NvO.

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:

- 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.

11.3. Other novelty considerations for PBOs for food and feed use

Where the PBO belongs to a species that has a history of safe food use, but the trait introduced is designed to allow marketing for food use of a part of the plant that does not have a history of continued use in the customary diet of a significant number of people in the UK or the EU beginning before 15th May 1997:

As there is no compositional data or experience of continued food use in the customary diet of a significant number of people in the UK or the EU to provide HSFU in support of the safety of these parts as food, the PBO requires a safety assessment of the new parts of the plant for use in food (see Section 15.2.1). This includes recommendations for new conditions of use (where relevant) prior to authorisation. This is required in particular to ensure that these parts of plants from non-PBO varieties do not enter the food chain.

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:

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 PBOs may require the use of a specific processing step to fully achieve the intended phenotype (for example, UV treatment – see Section 15.2.2); 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 15.2.2). Where a novel process is needed, this requires a Tier 2 FSA safety assessment under Other Safety Concerns (Section 15.2.2).

Where the trait introduced intends to biofortify the PBO with a substance previously exclusively used as a food 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 becomes a new dietary source for this substance previously provided in the form of supplements. This must be taken into consideration in the Tier 1 safety assessment in Sections 12.2 (Nutrition), 13.2 (Toxicity) and 14.2 (Allergenicity).

Where the PBO is 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.

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. All PBOs for feed use, whether they are from species with or without PFC, must undergo a Tier 1 safety assessment for the compositional and "Other Safety Concerns" criteria as described in Sections 12, 13, 14 and 15.

Assimilated Regulation (EC) No 767/2009 on the placing on the market and use of feed requires that representatives of the feed industry in Great Britain must be notified of new feed materials being placed on the market and that such materials must be 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.

12. Nutrition Tier 1 safety assessment

12.1. 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 as it is being consumed as food or feed at the date of the application in a way that is likely to be disadvantageous to the consumer." The nutritional quality of a PBO is defined as the contribution of food or feed consumed from the edible parts of the organism to human and/or animal health, including growth, maintenance and repair.

The Tier 1 safety assessment of Nutrition is used to determine whether the substances of interest present in the edible parts of the plant and identified in Section 8 are relevant for Nutrition. The following must be taken into consideration:

- Nutrient and antinutrient content.
- Digestibility.
- Differing nutritional needs for different populations and species.

Where the precision breeding intends to improve the nutritional quality of a PBO, applicants should identify both the expected beneficial effects and any potential safety concerns in their safety conclusion. Similarly, where possible negative effects on nutrition are expected from the genetic change, any mitigation factor removing the cause for safety concern should be identified. The Tier 1 nutrition assessment enables balanced consideration of beneficial and adverse changes.

12.2. 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 7. This means identifying any substances of interest (Sections 7 and 8) and determining their relevance and significance to nutrition. Answering the assessment question on Nutrition involves identifying the changes in nutritional quality and understanding their impact by comparison to a suitable benchmark reference. This section guides applicants through the steps outlined in Figure 7. Where substance(s) of interest are identified, Steps (**2**), (**5**) and (**6**) must always be considered regardless of the answers in previous steps. 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.

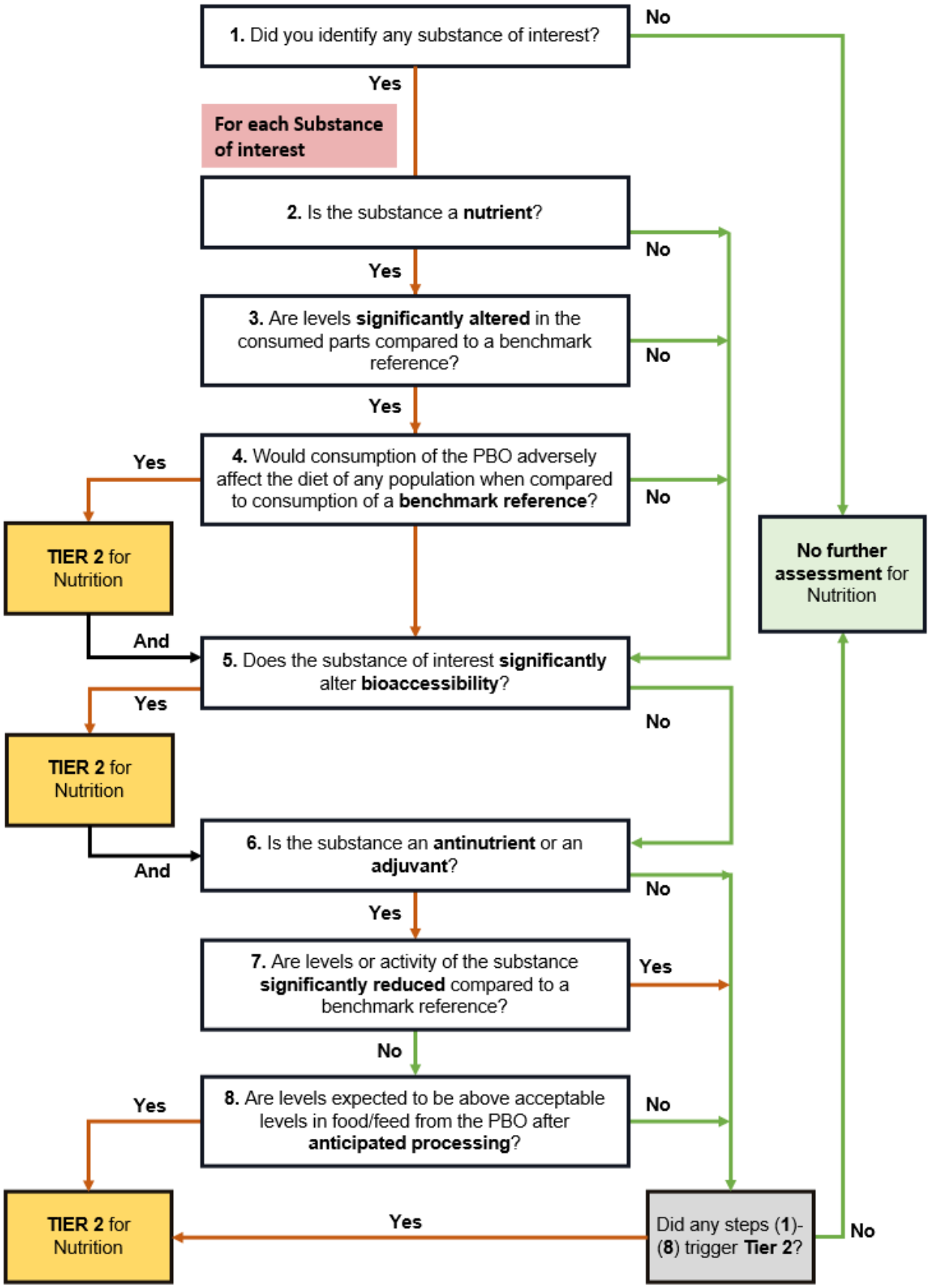


Figure 7.

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 reference varieties that have a HSFU/PFC in the UK or EU, and is biologically relevant to safety and quality. For detailed instructions, refer to Section 12.2.

Step (1) – Did you identify any substance of interest?

Substances of interest are the substances whose levels or activity is changed in the edible parts as a result of the phenotype or intended use, as identified in Section 8.

If the answer is No: This ends the Tier 1 safety assessment of Nutrition, no further safety assessment is needed. Proceed to Tier 1 safety assessment of Toxicity in Section 13.

If the answer is Yes: All steps of the Nutrition assessment shown in Figure 7 from Step (2) onwards must be completed for each identified substance of interest; Proceed to Step (**2**).

Step (2) – Is the substance a nutrient?

Nutrients include macro and micronutrients such as those described in Article 2(s) and Annex XIII of assimilated Regulation (EU) 1169/2011, that contribute to the nutritional quality of the edible parts of the PBO. Levels of nutrients in the edible parts of a plant may change because of changes in their production, transportation, accumulation or storage.

Particular attention should be given to substances in biological pathways related to Bioaccumulation. Applicants must also consider how any change to a biological transporter affects any other substrate in addition to the target substrate, as most transporters have more than one substrate.

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

If the answer is Yes: Proceed to Step (**3**).

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; 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 impact of increased zinc on nutritional quality.

Step (3) – Are levels significantly altered in the consumed parts compared to a benchmark reference?

Nutrient content is significantly affected if the quantity of a nutrient is reduced or increased, and the increase or reduction is beyond the range found in comparable TBOs. Alterations to levels of protein are only significant if the change to overall protein content in the consumed parts is beyond the range found in comparable TBOs.

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 11.3). 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.

Applicants must refer to an appropriate benchmark reference such as McCance and Widdowson (2021) to evaluate changes in nutrient quantity.

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

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

Step (4) – Would consumption of the PBO adversely affect the diet of any population when compared to consumption of a benchmark reference?

If a 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 (**3**) of the safety assessment for Toxicity (Section 13.2). 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 bioaccessibility of other nutrients. Similarly, if nutrient levels are decreased, applicants must determine whether any vulnerable populations may be 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. 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 National Diet and Nutrition Survey dataset (Public Health England, 2020). Applicants may also wish to consult relevant UK Scientific Advisory Committee on Nutrition (SACN) or Committee of Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) reports and position papers.

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 benchmark reference 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 on vitamins and minerals or COT reports.

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).

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 benchmark reference 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, 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 (**5**).

If the answer is Yes: The identified substance requires a Tier 2 FSA safety assessment for Nutrition. Proceed to Step (**5**).

Step (5) – Does the substance of interest significantly alter bioaccessibility?

Bioaccessibility is the proportion of nutrients that are available for absorption. An alteration to bioaccessibility is significant if it alters the proportion of any nutrient available for absorption within the edible food/feed parts when compared to an appropriate benchmark reference (as described in Section 9.1).

Bioaccessibility is affected by digestibility. Applicants must assess whether the substance of interest significantly alters the overall composition of the edible parts of the PBO in a way likely to significantly alter digestibility by comparing the composition of the edible parts of the PBO to an appropriate benchmark reference.

For macronutrients (for example, starch, carbohydrates, proteins and fat) applicants must also consider if the composition of the macronutrient is altered in a way likely to significantly alter digestion of the macronutrient when compared to an appropriate benchmark reference.

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

If the answer is Yes: A Tier 2 FSA safety assessment is required for Nutrition. Proceed to Step (**6**).

For example: A crop with a modified starch composition. A crop with comparable starch composition already on the market in the UK or EU and which meets the criteria for a suitable benchmark reference (Section 9.1) can be used to show that bioaccessibility is not significantly altered.

Step (6) – Is the substance an antinutrient or an adjuvant?

An antinutrient is any substance which interferes with the absorption of nutrients. An adjuvant is any substance which affects the activity of a nutrient or antinutrient.

If the species has a known antinutrient hazard, applicants must evaluate whether the substance of interest is likely to significantly alter the antinutrient content of the PBO.

Any expected 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.

If the answer is No: This ends the Tier 1 safety assessment for Nutrition for this substance. When all the substances of interest have been assessed for Nutrition, proceed to Section 12.3.

If the answer is Yes: Proceed to Step (**7**).

Step (7) – Are levels or activity of the substance significantly reduced compared to a benchmark reference?

Acceptable levels refer to levels/activity equivalent to or below those found in food/feed from a benchmark reference after the same anticipated processing. Applicants must compare levels/activity of the antinutrient to a benchmark reference using appropriate supporting evidence (references, test results, *etc.*).

If the answer is No: Proceed to Step (**8**).

If the answer is Yes: If all antinutrients are effectively removed or inactivated, this ends the Tier 1 assessment for Nutrition. When all the substances of interest have been assessed for Nutrition, proceed to Section 12.3.

Step (8) – Are levels expected to be above acceptable levels in food/feed from the PBO after anticipated processing?

In this section, 'acceptable levels' refers to levels/activity equivalent to or below those found in food/feed from a reference variety, after the same anticipated processing.

Applicants must identify the processing step(s) that remove or inactivate the antinutritional factor, if applicable, and evaluate the efficacy of antinutrient removal and/or inhibition using appropriate supporting evidence (references, test results, *etc.*).

If the answer is No: This ends the Tier 1 safety assessment for Nutrition for this substance. When all the substances of interest have been assessed for Nutrition, proceed to Section 12.3.

If the answer is Yes: The identified substance requires a Tier 2 FSA safety assessment for nutrition. This ends the Tier 1 safety assessment of Nutrition for this substance.

When all the substances of interest have been assessed for Nutrition, proceed to Section 12.3.

12.3. Conclusion of Tier 1 Safety Assessment for Nutrition

This ends the Tier 1 safety assessment of Nutrition:

- Where no substance of interest requires a Tier 2 safety assessment, no further assessment in needed on Nutrition.
- Where any substance of interest requires a Tier 2 safety assessment, further information may be required as part of the application, as described in Section 26.

Proceed to Tier 1 safety assessment of Toxicity in Section 13.

13. Toxicity Tier 1 safety assessment

13.1. 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."

The first step in the Tier 1 safety assessment of toxicity is to determine whether the substances of interest present in the edible parts of the plant and identified in Section 8. are relevant for toxicity.

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 interest relevant for toxicity of food and feed from 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 that are 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.

For the toxicity assessment, substances of interest 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 12 (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.

Precision breeding may be used to change both the levels of expression or the activity of a protein toxins (by altering its sequence / structure); therefore, the assessment is not limited to investigating changes in levels only, but also to ensure changes in activity are taken into account. Where applicants are uncertain about the safety of the new levels or new activity of a toxin in a PBO, it requires a Tier 2 safety assessment.

Bioaccumulation - Applicants must also consider the possibility of bioaccumulation of non-threshold toxic pollutants.

Substances with no HSFU – Where a substance has been introduced that was never present in food before, it requires a Tier 2 safety assessment. An existing protein intentionally altered by the genetic change is not considered as a substance with no history of consumption.

Variation in levels of substances of interest in the PBO must be understood in order to characterise possible effects on dietary exposure, **considering existing Health-Based**

Guidance Values (HBGVs) **as part of total intake** by humans (food) or by the target species (feed).

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.

13.2. 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 8. This means considering the changes of levels or activity of substances of interest identified in Sections 7 and 8. In navigating this safety assessment process, applicants must use the body of available scientific knowledge.

To answer the safety assessment question about Toxicity, levels of substances in the PBO should be compared to benchmark references that are suitable as described in Section 9.1; this includes for example, levels measured in a benchmark reference, levels listed in OECD consensus documents, levels listed in McCance and Widdowson dataset.

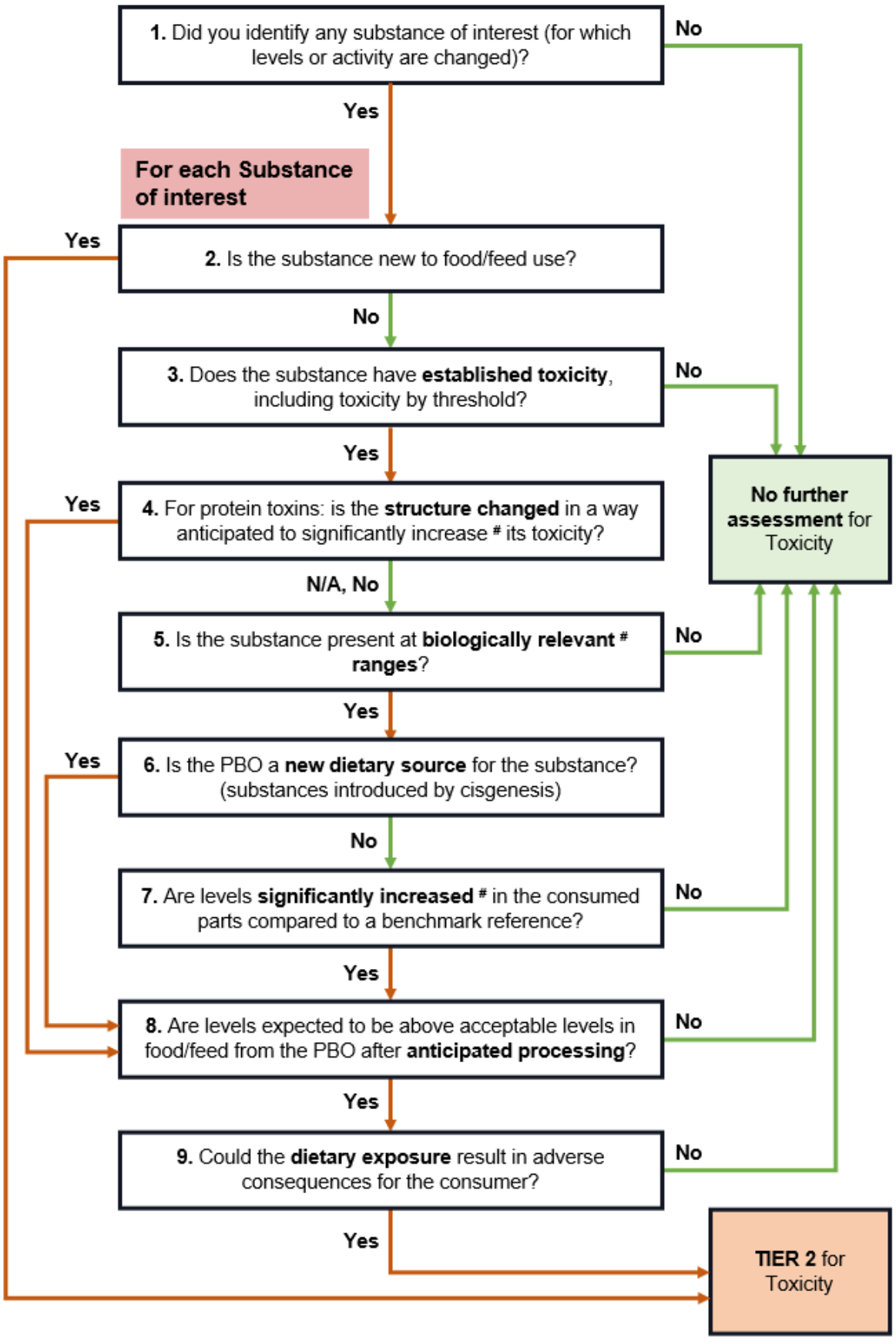


Figure 8.

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?**" Substances of

interest are substances which levels or activity are changed in edible parts, identified as part of the phenotype of the PBO and its intended use (Section 8). A change in levels 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 reference variety that have a HSFU or PFC. A change in activity is significant if there is robust evidence of a change in potency. **#** Steps (**4**), (**5**) and (**7**), where there is an intended decrease in the production or activity of a toxin, consider the implications on how the PBO is processed or consumed in Sections 15.2.2 and 15.2.3. For detailed instructions, refer to Section 13.2.

Step (1) – Did you identify any 'substance of interest' (for which levels or activity are changed)?

Substances of interest are the substances whose levels or activity is changed in the edible parts as a result of the phenotype or intended use, as identified in Section 8.

Because toxins are frequently linked to stress-response, applicants should have considered if their PB trait is intended to significantly alter growth of the plant. When cisgenesis or intragenesis is used, identifying substances with known toxicity (and particularly toxins) expressed in the donor but not in the host can contribute to the hypothesis-driven identification of toxic functions normally silent in the host that could be restored/activated upon introduction of some cisgenes.

Examples of mechanisms by which the genetic change(s) may indirectly 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, 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.
- 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.

If the answer is No: 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 14.

If the answer is Yes: All steps of the Toxicity assessment shown in Figure 8 from Step (2) onwards must be completed for each identified substance of interest, as relevant; Proceed to Step (**2**).

Step (2) – Is the substance new to food/feed?

Where a substance produced is **new to the diet**, the analysis of its toxicity should be reviewed as part of a Tier 2 safety assessment. This is due to the absence of any HSFU

or PFC of this substance. Note that proteins targeted by the genetic change are excluded from this category of substances new to the diet.

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

If the answer is Yes: The identified substance requires a Tier 2 FSA safety assessment for toxicity. This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3.

Step (3) – Does the substance have established toxicity, including toxicity by threshold?

Information to understand if the identified substance/protein can be toxic includes: databases of toxicity data on chemicals and tools for the prediction of the toxicity of chemicals, public reference databases identifying key plant toxicants (such as the OECD Consensus documents for major crop species (OECD, live database), EFSA Compendium of Botanicals (2012)), peer reviewed scientific literature, proprietary phenotypic or toxicology data.

Toxic properties are considered established if the substance is:

- **A natural toxins -** Naturally occurring toxins (hereafter referred to as 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. 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.

  Identification of toxic protein can also be supported by a sequence homology analysis (for example BLAST searches) of the encoding gene with an available annotated database (for example experimentally verified entries in GenBank, UniProt, String, EMBL-EBI).

  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.

- **A substance with a well-known, documented toxicity by threshold -** Particular attention should be given to: Secondary metabolites (for example anthocyanins)

also produced by plants as part of protection mechanisms against abiotic stress but not identified as natural toxins *per se*; Nutrients (when they are the target of the precision breeding) and their precursors or degradation products which may have a well-known, documented toxicity by threshold (for example some vitamins).

Examples of substances of interest relevant to food/feed toxicity, other than natural toxins:

- The intention of the change is to increase the levels of a vitamin. It is well established in the literature that this vitamin is one of the fat-soluble vitamins known to have potential toxicity: the vitamin is a substance of interest relevant for the toxicity assessment.
- The intention of the change is to increase the levels of a vitamin; it is reasonably anticipated this results in changes in levels of related substances, in particular precursor and degradation product: where these related substances have documented toxicity, they are substances of interest relevant for the toxicity assessment.
- The intention of the change in a crop is to allow tolerance to environmental stress, and as a consequence to increase yield. Growth of this crop in abiotic stressful conditions is well documented to result in the increase in levels of a range of secondary metabolites. Of these secondary metabolites, only the ones that have documented toxicity are substances of interest relevant for the toxicity assessment.

If the answer is No: This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3.

If the answer is Yes: Compositional data on the substance must be used to support the Tier 1 safety assessment for toxicity; proceed to Step (**4**).

Step (4) – For protein toxins: Is the structure changed in a way anticipated to significantly increase its toxicity?

This step is relevant solely for protein toxins targeted by the genetic change. Alteration in the sequence of a toxin has the potential to increase its toxicity. The conclusions on the potency must be based on robust evidence from the scientific literature, or where available, proprietary phenotypic or toxicology data.

The resulting new amino acid sequence should be considered for any change in toxic motifs and the potential consequences to toxicity (as reviewed by Palazzolo *et al.*, 2020, 2024). The resulting new amino acid sequence can be analysed by comparison (BLAST, HMM profile alignment) with its parent and with an available annotated database (for example GenBank, UniProt, String, EMBL-EBI), or through the use of *in silico* toxicity prediction tools (for example ToxinPred2, Toxify).

If the answer is No, or if the substance is not a protein toxin: Proceed to Step (**5**). To note, only increases in the toxicity of a protein toxin are a concern for the toxicity of food/feed. Where the intent of the change in sequence is to decrease the toxicity of

the toxin, the implications on how the PBO is processed (Section 15.2.2) or how it is consumed (Section 15.2.3) must be considered.

If the answer is Yes: An analysis of the new amino acid sequence of the protein targeted by the genetic change must be used to support the Tier 1 safety assessment for toxicity; proceed to Step (**8**).

Step (5) – Is the substance present at biologically relevant ranges?

The substance is not relevant for the toxicity of food/feed if applicants can demonstrate that it does not have any adverse effect at the levels expected to enter the food or feed chain; 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.

When determining the biological 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.

If the answer is No: This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3. Where the intent of the change is to decrease the levels of substances with established toxicity, the implications on how the PBO is processed (Section 15.2.2) or how it is consumed (Section 15.2.3) must be considered.

If the answer is Yes: Proceed to Step (**6**).

Step (6) – Is the PBO a new dietary source for the substance?

Where any substance(s) produced are **new to the organism**, they must be included for consideration in Steps (**8**) and (**9**) 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 (**7**).

If the answer is Yes: Proceed to Step (**8**).

Step (7) – Are levels significantly increased in the consumed parts compared to a benchmark reference?

Each different part of the plant intended for consumption must be considered separately. Benchmark references are identified in Section 9.1, they must represent the ranges normally found in the consumed part.

Where a change in levels is intended, the statistical analysis **must** use proprietary compositional data on the substance (as described in Section 9.2).

For reasonably anticipated, not intended changes in levels, where no quantitative information is available, an equivalent trait in a suitable benchmark reference may

allow the conclusion that the levels are not significantly increased, if the equivalent trait is already commonly available on the food and feed market in the UK/EU and if the reference variety shares the same role in the diet as the PBO and is from a species with a HSFU/PFC in UK or EU.

If the answer is No: This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3. To note, only increases in the levels of a substance are a concern for the toxicity of food/feed. Where the intent of the change is to decrease the levels of substances with established toxicity, the implications on how the PBO is processed (Section 15.2.2) or how it is consumed (Section 15.2.3) must be considered.

If the answer is Yes: Proceed to Step (**8**).

Step (8) – Are levels expected to be above acceptable levels in food/feed from the PBO after anticipated processing?

Substance levels 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 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 acceptable 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.

When a protein toxin is anticipated to exhibit increased toxicity as a result of a change in sequence / structure, applicants must take into account that reduction of levels as a result of processing may not provide sufficient reassurance on the residual toxicity in the processed food /feed.

Decision-making on this relies on the identification of processing steps (together with their efficacy) by which the 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. This must consider potential recent novel uses from whole, parts or extracts from organisms.

If the answer is No: This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3. 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, a Tier 2 safety assessment is required so that appropriate recommendations for conditions of use can be made (see Section 15.2.1).

If the answer is Yes: Proceed to Step (**9**).

Step (9) – Could the dietary exposure result in adverse consequences for the consumer?

Where levels of substance(s) 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, the toxicity of the PBO is judged significantly increased 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, and HBGVs or TTCs of the substance(s) of interest.

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 (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 No: This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3.

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. This ends the Tier 1 safety assessment of Toxicity for this substance. When all the substances of interest have been assessed for their toxicity, proceed to Section 13.3.

13.3. Conclusion of Tier 1 Safety Assessment for Toxicity

This ends the Tier 1 safety assessment of Toxicity:

- Where no substance of interest requires a Tier 2 safety assessment, no further assessment in needed on Toxicity.
- Where any substance of interest requires a Tier 2 safety assessment, further information may be required as part of the application, as described in Section 27.

Proceed to Tier 1 safety assessment of Allergenicity in Section 14.

14. Allergenicity Tier 1 safety assessment

14.1. 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.

Allergenic properties of feed are often considered as part of toxicity in animals. This includes consideration of PBOs for use as pet food. In line with common farming practices, allergenicity safety concerns related to feed can be identified using the toxicity Tier 1 assessment described in Section 13, but the conclusions and their implications for allergenicity must be used in this section to determine whether the criterion set in Regulation 20 (1) (c) (iii) is met.

The overall allergenicity of any food or feed is determined by the levels of clinically relevant allergens or their structure and may be altered if these are significantly affected by the genetic change. In some instances (for example, for gluten), the food allergenicity is not a function of a single substance but instead results from multiple copies of multiple allergens; in such cases, the allergenicity will be determined by the significance of the change to the overall group of allergens, rather than to the individual allergens. The steps described in Section 14.2 are designed to determine whether the allergenicity of any food or feed can be expected to be altered as a result of the genetic change and so whether a Tier 2 safety assessment is required. **There is a very low probability that alteration of the structure of a protein, or very significant increase in the levels of a known allergen with low prevalence, potency or severity, would make proteins become clinically relevant for allergenicity. However, should applicants have clear reasons to believe that the genetic change in their PBO raises this possibility, they should submit the targeted proteins to Steps (4) and (5) of the below assessment respectively.**

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 significantly affects the structure or levels of endogenous or intentionally introduced allergens, so that it could alter allergenicity.

14.2. 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 9.

This guidance document provides further information on:

- Organisms which are of allergenic concern.
- Relevant allergenic proteins.
- The definition of significance regarding changes to allergen quantity and structure.
- Methodology to be used for quantification of allergenicity, where relevant; and
- Principles to be followed for data submission.

The FSA requires two assurances on allergenicity for marketing PBOs for consumption:

1. That there is no significant increase to the quantity, or significant change in the structure of a known allergenic protein in the consumed parts of a PBO which may significantly alter allergens in the produced food/feed and so alter its overall allergenicity.

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 benchmark references (as defined in Section 9.1). Different benchmark references may be selected for different purposes and may include a TBO variety of the same species that has a HSFU and for which the potential to induce an allergenic response is understood.

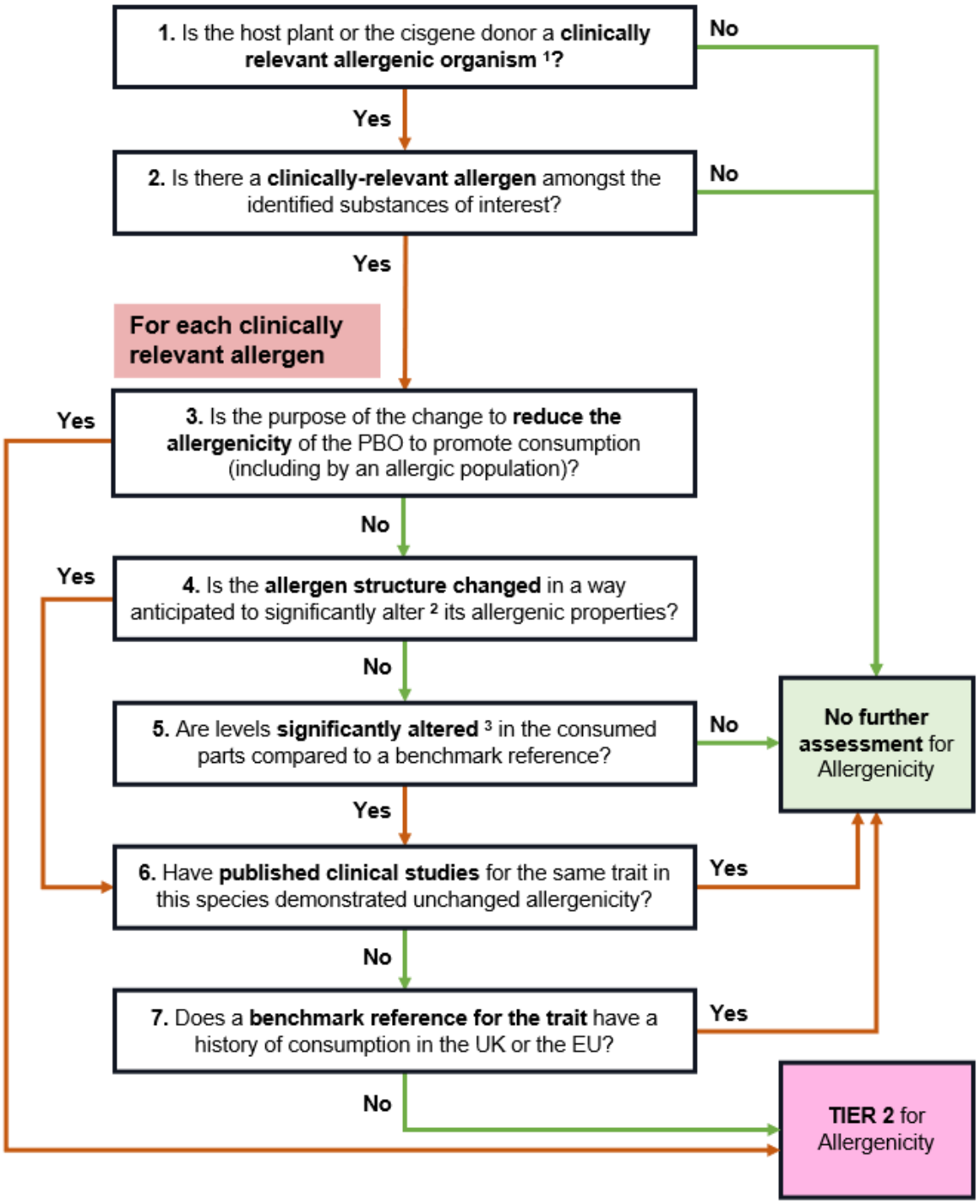


Figure 9.

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?**" Step (**1**), note **1**: Where there are clear reasons to believe that the genetic change to a non-clinically relevant allergenic organism may result in a protein becoming clinically relevant for allergenicity, this protein will need to undergo the assessment described in Steps (**4**) and (**5**). Steps (**4**), note **2**: a change in allergenic protein structure is significant where a credible risk hypothesis can be drawn from robust scientific evidence that the change may affect allergenicity. Steps (**5**), note **3**: A change in levels of an allergen is significant when the levels are increased beyond the ranges found in reference food composition datasets by an order of magnitude (≥ x10), or outside the ranges

found in suitable reference variety that have a HSFU in the UK or EU by an order of magnitude (≥ x10), and the increase may be biologically relevant to safety. For detailed instructions, refer to Section 14.2.

Step (1) – Is the host plant or the cisgene donor a clinically relevant allergenic organism?

Organisms containing common food allergens are of public health importance if:

- They are subject to mandatory labelling listed in Annex II of assimilated Regulation 1169/2011 on food information to consumers.
- They raise a high allergenic concern in the UK or EU due to significant allergenic prevalence, potency, and severity, as established in the scientific literature.

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).

For example: an applicant alters a pathway related to an agronomic trait. There is no clear scientific evidence or consensus that this change would alter levels of allergens within the PBO. The applicant answers 'no' to Step (**1**).

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 15.

If the answer is Yes: Proceed to Step (**2**).

Step (2) – Is there a clinically-relevant allergen amongst the identified substances of interest?

Substances of interest are the substances whose levels or activity is changed in the edible parts as a result of the phenotype or intended use, as identified in Section 8.

A protein is a clinically relevant allergen when there is robust scientific evidence in the peer reviewed scientific literature that it is linked to adverse allergenic outcomes. Databases of allergenic proteins may also be useful to consult for information on allergenic proteins within the PB organism(s).

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/

Where databases are consulted and the match is partial, scientific literature may confirm the allergenicity of the protein. Where there is no evidence that a protein has clinical relevance for allergenicity, answer 'no' to Step (**2**).

Because allergenic proteins are frequently linked to stress-response, applicants should consider if their PB trait is intended to significantly alter growth or storage conditions of the plant (Section 6.5).

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 15.

If the answer is Yes: All steps of the Allergenicity assessment shown in Figure 9 from Step (3) onwards must be completed for each identified clinically-relevant allergen of interest, as relevant. Compositional data on the identified allergen(s) must be used to support the Tier 1 safety assessment for allergenicity; process to Step (**3**).

Step (3) – Is the purpose of the change to reduce the allergenicity of the PBO to promote consumption (including by an allergic population)?

Decreasing the levels of an allergen or altering its structure so that its allergenic properties are decreased can reduce the allergenicity of a crop.

When the purpose of altering a crop is to reduce its allergenicity in order to promote safe consumption, including by an allergic population, the claim should be supported by clinical studies considering the potential to elicit a reaction in sensitive people. A pre-existing published clinical study of the same trait may support assessment, however the evidence should always be reviewed as part of a Tier 2 safety assessment.

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

If the answer is Yes: The claimed decrease in allergenicity of the PBO with respect of the identified allergen requires a Tier 2 FSA safety assessment for allergenicity. This ends the Tier 1 safety assessment of Allergenicity for this substance. When all the clinically-relevant allergens of interest have been assessed for their allergenicity, proceed to Section 14.3.

Step (4) – Is the allergen structure changed in a way anticipated to significantly alter its allergenic properties?

This step is relevant solely when the sequence of an allergen is targeted by the genetic change. Alteration in the sequence of an allergen has the potential to alter its allergenic properties.

A change in allergen structure is significant if it is expected to alter the allergenic activity of the protein in such a way that it can be reasonably anticipated to alter allergenicity for consumers. The resulting new amino acid sequence should be considered for significant changes in allergen structure, especially those impacting binding sites.

The conclusions on whether the change is likely to be significant and so potentially impact consumers must be based on robust evidence from the scientific literature, structural modelling, or where available, proprietary phenotypic information or clinical data. Applicants are not expected to generate data to determine significance. In some instances, prior experience of the crop may constitute sufficient evidence.

The resulting new amino acid sequence can be analysed by comparison with its parent and with an available annotated database, or through use of *in silico* prediction tools.

Alignment tools can include: BLAST, HMM profile alignment.

Databases can include: GenBank, UniProt, String, EMBL-EBI

In silico tools can include: https://alphafold.ebi.ac.uk/

For example:

A substantial alteration of the domains which cross-link to IgE receptors – Where use of *in silico* tools and scientific literature demonstrate strong evidence that this change could greatly increase binding affinity, therefore potentially increasing the severity of a clinical allergic response in consumers, the applicant would answer 'Yes' to Step (**4**).

A slight alteration of an allergenic protein structure to generate an improved phenotype - Where *in silico* tools and scientific literature do not show evidence that this change could substantially impact protein activity in allergenic responses, the applicant would answer 'No' to Step (**4**).

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

If the answer is Yes: Proceed to Step (**6**).

Step (5) – Are levels significantly altered in the consumed parts compared to a benchmark reference?

A change in levels of allergens is considered significant if the levels of protein in the edible parts is expected to be outside the ranges found in equivalent TBOs **by an order**

of magnitude (≥ 10 fold difference compared to a benchmark reference) (Houben *et al.*, 2020).

The applicant may utilise proprietary scientific evidence obtained during the development of the PBO, robust prior experience with the crop and the trait, and peer-reviewed scientific literature to conclude whether the change is likely to be significant and so potentially clinically impact consumers.

For example:

- Changes to a trait confined to leaf tissue will not be relevant to allergenicity if only the fruit is consumed. The applicant answers 'No' to Step (**5**).

- An allergen is increased within consumed parts to increase overall protein content and improve desirable characteristics. The intention is to increase protein content by approximately 20%, and prior breeding experience in addition to searching the scientific literature for the target gene does not indicate that the phenotype associated with the genetic change would significantly increase protein (by an order of magnitude). The applicant answers 'No' to Step (**5**).

If the answer is No: This ends the Tier 1 safety assessment of Allergenicity for this substance. When all the clinically-relevant allergens of interest have been assessed for their allergenicity, proceed to Section 14.3.

If the answer is Yes: Proceed to Step (**6**).

Step (6) – Have published clinical studies for the same trait in this species 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 change in allergenic response is observed when compared to a suitable benchmark reference (generally, the progenitor species).

If the answer is Yes: This ends the Tier 1 safety assessment of Allergenicity for this substance. When all the clinically-relevant allergens of interest have been assessed for their allergenicity, proceed to Section 14.3.

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

Step (7) – Does a benchmark reference for the same trait belonging to the same species have a history of consumption in the UK or the EU?

This question is intended for PBOs where the genetic change may impact allergens, and the change has been made to generate a genomic sequence which is comparable to a traditionally bred variety already commonly available on the food and feed market.

For example: to minimise linkage drag, such as transferring a desirable trait present within an exotic variety to 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 food and feed market demonstrating the similarity of the genetic change; and, robust prior experience with the crop and the trait, and/or available peer-reviewed scientific literature demonstrating HSFU of the trait in the EU/UK with the comparable genetic change.

The genetic sequence used as a reference must be within the primary gene pool of the PBO. Applicants must utilise robust scientific evidence that the genotype and the trait of the benchmark reference is already commonly available on the food and feed market in the UK/EU to answer this question.

For example: a genetic change is made to an allergenic protein to improve the quality of a desirable trait in food product. This same genetic change is present in other varieties of the same crop which are already commonly available on the market in the UK/EU. The applicant would answer 'Yes' to Step (**7**).
For example: a genetic change is introduced which impacts an allergenic protein and results in a similar phenotype as another crop variety. However, the PB genetic change targeted a completely different pathway to that within the variety already commonly available on the market in the UK/EU. The potential hazards will be different, and therefore the applicant would answer 'No' to Step (**7**).

If the answer is Yes: This ends the Tier 1 safety assessment of Allergenicity for this substance. When all the clinically-relevant allergens of interest have been assessed for their allergenicity, proceed to Section 14.3.

If the answer is No: The anticipated change in allergenicity for the identified allergen requires a Tier 2 FSA safety assessment for allergenicity. This ends the Tier 1 safety assessment of Allergenicity for this substance. When all the clinically-relevant allergens of interest have been assessed for their allergenicity, proceed to Section 14.3.

14.3. Conclusion of Tier 1 safety assessment for Allergenicity

This ends the Tier 1 safety assessment of Allergenicity.

- Where no clinically relevant allergen of interest requires a Tier 2 safety assessment, no further assessment in needed on Allergenicity.
- Where any clinically relevant allergen of interest requires a Tier 2 safety assessment, further information may be required as part of the application, as described in Section 28.

Proceed to Tier 1 safety assessment of Other Safety Concerns in Section 15.

15. Other Safety Concerns

15.1. 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. As with Sections 11-14, assessment of other safety concerns is hypothesis driven and limited to changes for which there is a sound scientific rationale to believe the product may present safety concerns, based on the available knowledge. Applicants are not expected to conduct non-hypothesis based searches or assays. Concerns to declare in the "Other Safety Concerns" category are any traits which could foreseeably affect the safety of any food or feed produced from the PBO which are not required to be declared by virtue of other sections of this guidance.

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 11 (Novelty), 12 (Nutrition), 13 (Toxicity) and 14 (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 15.2 is yes, a Tier 2 FSA safety assessment is required (Figure 10).

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

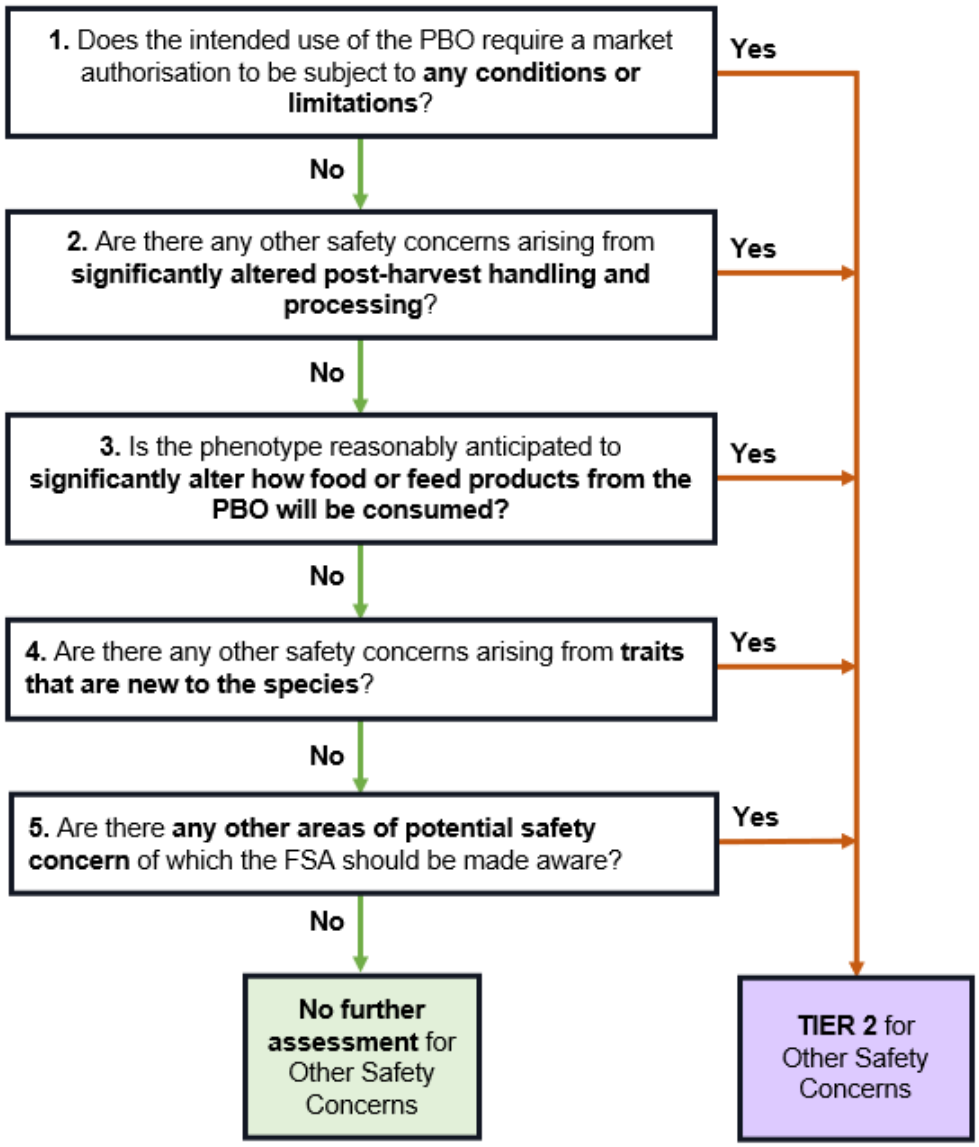


Figure 10.

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?**" This should be used as a guide to 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. For detailed instructions, refer to Section 15.2.

15.2.1. Step (1) – Does the intended use of the PBO require a market authorisation to be subject to any conditions or limitations?

The Secretary of State must consider whether a new marketing authorisation should be subject to any conditions or limitations under Regulation 30 (2). Market authorisation may be subject to conditions or limitations in the following circumstances:

Restrictions of use - 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 these would be safe to use when such restrictions 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.

New parts for food use – When the PBO belongs to a species that has a history of safe food use, but the trait introduced is designed to allow marketing for food use of a **part of the plant** that does not have a history of continued use in the customary diet of a significant number of people in the UK or the EU beginning before 15th May 1997, there is no compositional data and experience of continued food use to provide HSFU in support of the safety of this part as food. The PBO requires an appropriate safety assessment of the use in food of the new part of the plant. Recommendations for new conditions of use prior to authorisation are required in particular to ensure that the same part of the plant, but which does not have the trait introduced in the PBO, does not enter the food chain. A Tier 2 safety assessment is needed.

For example:

A part of the PBO is no longer toxic – The change removes a toxicant from a part of the plant which historically prevented its safe consumption.

A part of the PBO has been made more palatable – The change makes part of the PBO more desirable as a food, and the intention is to market this part of the PBO as food, where previously it was rarely eaten (therefore there is no history of consumption in the customary diet of a significant number of people in the UK or EU beginning before 15th May 1997).

PBOs for feed use only - 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).

Biofortified PBOs – Where nutrient content is intentionally and significantly increased in a PBO, an application under Regulation 22 must be made, to review how the nutrient increase will be communicated throughout the food/feed supply chain and provide transparency for consumers and food/feed business operators. Management of concomitant consumption of the same nutrient from multiple dietary sources, including from food/feed additives, should also be considered, particularly as biofortified products become diversified.

After market-authorisation of the PBO - 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.

15.2.2. Step (2) – Are there any other safety concerns arising from significantly altered post-harvest handling and processing?

Changes to post-harvest handling and processing are significant if there are major differences to conventional practices, and there is a reasonable expectation that the changes may raise safety and/or quality concerns based on a sound scientific rationale. This 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 handling (for example, storage).

Applicants are expected to have sufficient knowledge of the anticipated processes or storage practices (including key food safety measures such as microbiological control measures) in order to consider the impact of their alteration on food safety and/or quality where:

- The **intention** of the change is to alter processing or storage conditions.
- The introduced trait can be **reasonably anticipated** to lead to a **significant change** in a processing step or in storage, as has been seen with similar traits already in the food chain.

The impact of the above changes in processing or storage must be assessed with reference to relevant peer reviewed research or existing traits as benchmark reference (see Section 9.1) for the following:

- For processing: content/bioaccessibility of a nutrient; removal/inactivation of antinutrients, toxicants or microbial contaminants.
- For handling, including extended periods of storage: increase in levels of toxicants (for example some secondary metabolites), of clinically relevant allergens or of microbial contaminants; decrease of nutritional quality.

For example:

New processing phenotype – The phenotype makes it possible for a plant which must usually be cooked prior to consumption to be eaten raw. Not cooking removes a Critical Control Point used to reduce microbiological hazards in addition to controlling the levels in toxic substances.

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, allergens. For example, the phenotype of a fruit containing clinically relevant allergens allows its storage for month instead of days. The scientific literature presents strong evidence that prolonged storage of the fruit can significantly increase the clinically relevant allergens.

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 11.3.

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.

15.2.3. Step (3) – Is the phenotype reasonably anticipated to significantly alter how food or feed products from the PBO will be consumed?

A change in consumption is significant if there is a major intended or reasonably anticipated change in the quantity to be consumed or in the populations likely to consume the food/feed from the PBO, and there is a reasoned hypothesis that the change may raise safety and/or nutritional quality concerns. Any such concern requires an FSA Tier 2 safety assessment.

A significant change in consumption could result from the trait where:

- The **intention** of the trait is to significantly alter how the PBO will be consumed, such as through intentionally improving palatability, enabling new consumption by a vulnerable group, or providing a specific health benefit.
- The introduced trait can be **reasonably anticipated** to lead to a significant change in how the PBO will be consumed, as has been seen with similar traits already in the food chain.

Examples of phenotypes associated with a significant change in consumption, and for which impact on the diet and on safety must be considered:

– Increased palatability of a crop achieved by significantly reducing fibrous content, thereby making it easier to chew and enabling consumption in larger quantities or by new populations.

– A nut has been precision bred to grow without shell, this makes it an easier food to eat on the go and likely to be eaten in larger quantities as a consequence. The nut is naturally rich in selenium and increase in consumption is a safety concern.

15.2.4. Step (4) – 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?

15.2.5. Step (5) – Are there any other areas of potential safety concern of which the FSA must be made aware?

While this guidance aims to capture a wide range of safety concerns and to guide applicants through a safety assessment, it is not possible to anticipate all traits and resulting phenotypes which may be achievable by precision breeding. Some safety concerns that are not otherwise identified by applicants by taking the steps outlined in this guidance may be foreseeable by applicants based on the well-established scientific knowledge. These types of concerns should be outlined in applications.

Are there any gaps in knowledge or methodological uncertainties that have a reasonable prospect of impacting the conclusion that the genetic change does not introduce any additional features that may affect the safety of any food or feed produced from the PBO?

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

15.3. Conclusion of Tier 1 assessment for Other Safety Concerns

This ends the Tier 1 safety assessment of Other Safety Concerns.

Where the answer to all questions in Section 15.2 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 3: No further safety assessment is required for Other Safety Concerns.

Where the answer to any question in Section 15.2 is 'Yes': A Tier 2 safety assessment is required, further information is required as part of the application, as described in Section 29.

16. Information to be provided following Tier 1 safety assessment

Part 4 identifies the information to be provided for all criteria, to summarise the applicant's Tier 1 safety assessment; this information must be included whether a Tier 1 safety assessment is sufficient or whether an additional Tier 2 safety assessment is needed.

Part 5 identifies the additional information that needs to be provided specifically to support the Tier 2 safety assessment of the criteria for which it was identified a Tier 1 safety assessment was not sufficient. No additional information is needed for the criteria for which a Tier 1 safety assessment is sufficient.

Part 4 - Information on Tier 1 applicant-led safety assessment to include in all 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". Each of the steps described in the Part 3 flowcharts represent 'key considerations' for the Tier 1 assessment by applicants. Where applicants identify additional factors which affect their safety assessment, these must be taken into account in the Tier 1 safety assessment and reported as part of the information included in the application.

There are two application 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. The information identified in this section must be provided for applications under both Regulation 20 and Regulation 22.

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.

Where applicants use commercially sensitive data to demonstrate safety, a brief summary of this data must be provided to the FSA, but applicants can request for this data and any summary relating to it to be treated as commercially confidential (Regulation 34).

17. General considerations on the information to include on Tier 1 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. 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.

Details on the types and sources of data to be used are provided in the corresponding sections of the guidance. Datasets, including sequence data, are not required to be provided when a Tier 1 safety assessment is sufficient, 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.

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).

The information required from the Tier 1 safety assessment also needs to be provided as the starting point for a Tier 2 safety assessment. While data on variations in levels of substances used to inform a Tier 1 assessment may not be required to be submitted, 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.

17.1. Information to provide on substances of interest submitted to Tier 1 safety assessment

- **Identification of the substances of interest:** For non-proteins, colloquial and IUPAC names; for proteins, reference to the database entry in UniProt or GenBank, or similar where available.

  Where it is known, a brief description of the **mechanism** by which the genetic change(s) alter the levels or the activity of the substance of interest should always accompany its identification. For example, 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).

17.2. Information to provide when conclusion on the Tier 1 safety assessment involves compositional analysis

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

- **Details on published data used as benchmark reference:** Brief description and reference; rationale for the selection of the data (see Section 9.1), or

  Identification of the reference variety from a species with HSFU/PFC: Taxonomic information, including variety where relevant; why it is concluded that the role in the diet is similar; rationale for the selection as a reference (see Section 9.1).

- **Proprietary compositional information on the substances of interest in the PBO:** Number of representative samples used (a minimum of 5 representative samples independently harvested should be used); brief description of the sampling plan (criteria for selecting the sampling sources, how they ensure representativeness of crop compositional variability), of the source (the geographical origin of the crop used to provide the samples must be specified) (See Sections 9.2, 9.3); mean and standard deviation for the PBO and for its benchmark reference; the part of the plant it relates to must be specified; the statistical methods used (for example unpaired T-test, Anova), and results of the statistical analysis must be specified.

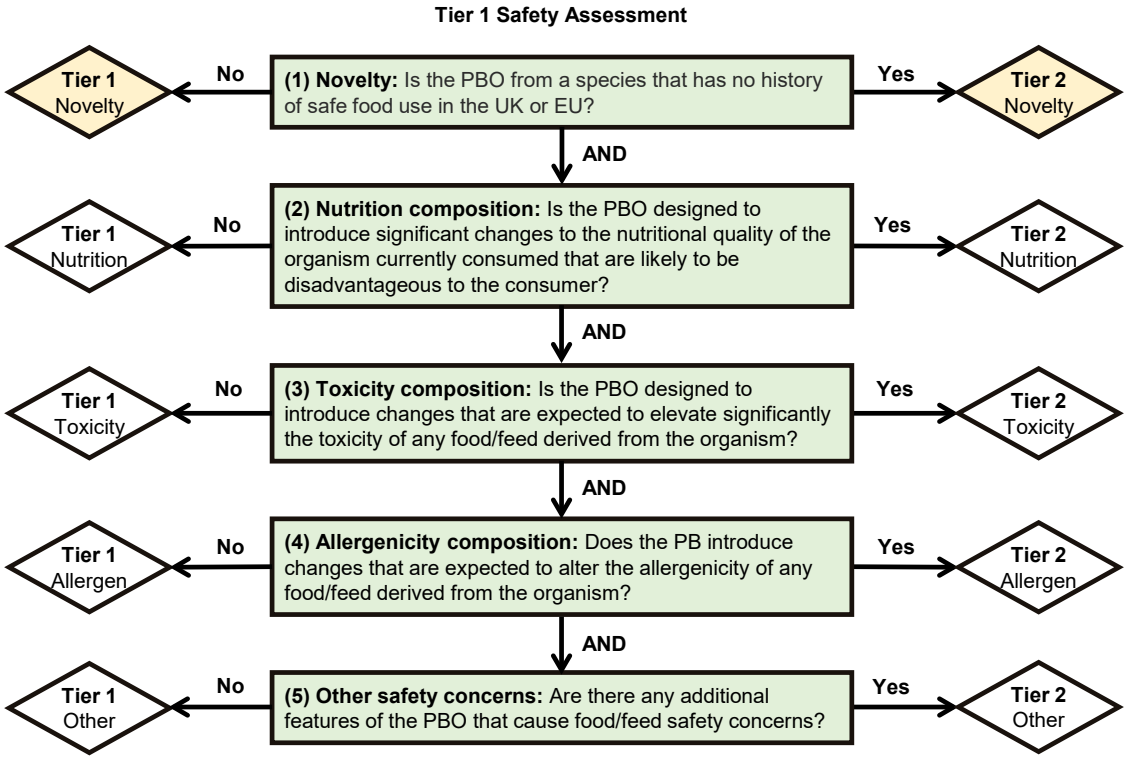
17.3. Information to provide when conclusion on the Tier 1 safety assessment involves using trait as a benchmark reference

Using a trait as a benchmark reference must always be supported by a **strong rationale** addressing the following points:

- **Taxonomic information**, including variety where relevant, of the TBO exhibiting the trait; rationale justifying the selection of a related species or of a species from the same family where relevant (see Section 9.1).
- **Summary of similarity of the trait** (brief description of the genotype or mechanism of the trait), how it was obtained.
- **Summary of how long the trait has been available in the food chain** - the trait must already be commonly available on the market in the UK/EU.
- **Why it is concluded that the role in the diet is similar** - this may quote consumption databases such as the EFSA Comprehensive Food Consumption Database (2018), or the Public Health England NDNS dataset (2020).

For example:
- The trait is resistance to a pest; this can be the result of a change to the target of the pest (limited effect on composition), or this could be achieved by changes to the structural defences (possible effect on accessibility and digestibility).

18. Information on Novelty to include 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:

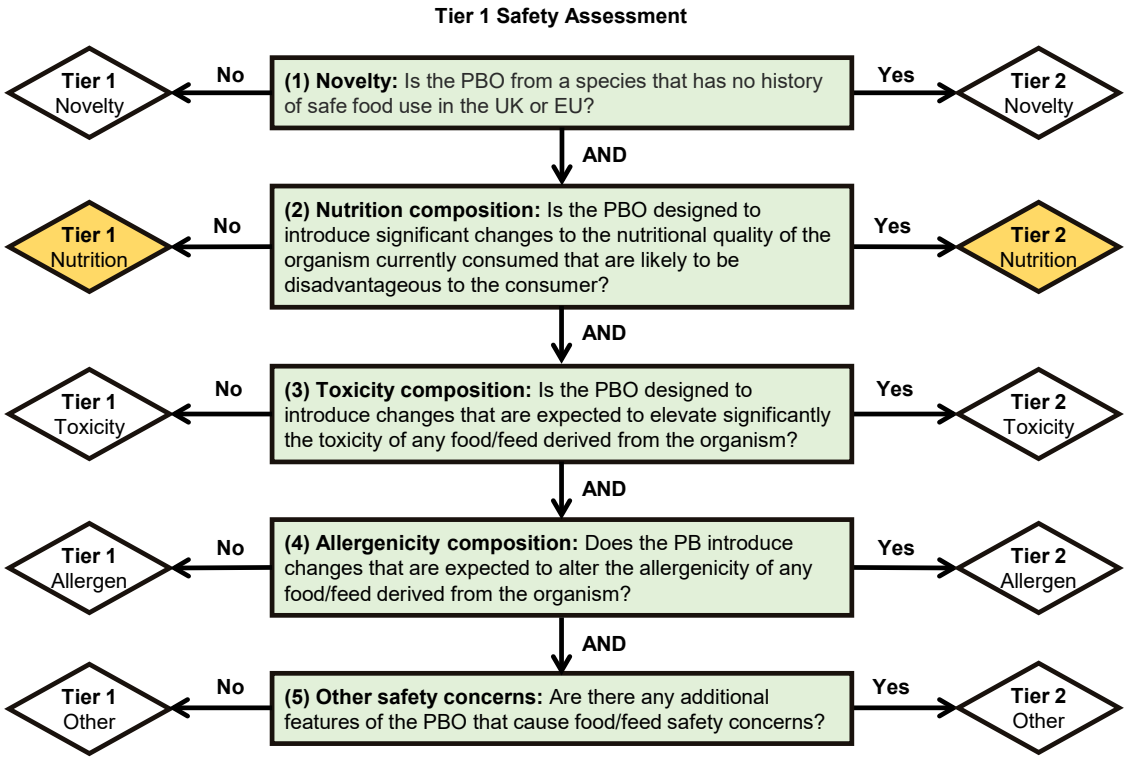
- **A statement concluding on the presence or absence of HSFU**; the history of safe food use within the UK or EU prior to 15 May 1997 must relate to how the PBO is intended to be used as a source of food, note that there might be different histories of consumption for different parts of the organism.

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. This may refer to evidence that crops are commonly grown or used in the EU or UK (seed registers, official agricultural statistics, importation data, EU Novel Food status catalogue).

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 15).

19. Information on Nutrition to include in all applications



19.1. State whether any substance of interest was identified in the PBO

If no, provide:

- Statement of confirmation that no substance of interest was identified from considering the phenotype resulting from the genetic change(s), and there is no nutritional safety concern for the PBO.

  No further information is needed on the Tier 1 Nutrition safety assessment.

If yes, follow the instructions in Sections 19.2 to 19.8 **for each identified substance of interest**. The submission should consist of individual summaries of the assessment of each substance of interest, and a final overall conclusion to the Tier 1 Nutrition assessment.

19.2. State whether the substance of interest is a nutrient

If no, provide:

- Statement confirming the substance of interest is not a nutrient.

  Proceed to Section 19.5.

If yes, provide:

- Statement confirming the substance of interest is a nutrient. Include a brief description of the nutrient role in nutrition.

  Proceed to Section 19.3.

19.3. State whether levels are significantly altered in the consumed parts compared to a benchmark reference

If no, provide:

- Statement of confirmation that levels are not significantly different from a benchmark reference. This must describe how levels compare with those found in a reference variety from a species with HSFU, including statistical analysis, using proprietary compositional information on the substance of interest in each part from the PBO destined for food or feed use; this may refer to typical range found in equivalent TBOs. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.

  Proceed to Section 19.5.

If yes, provide:

- Summary of the analysis comparing the levels of the substance in each part from the PBO destined for food or feed use with those found in a reference variety from a species with HSFU, including statistical analysis. This must use proprietary compositional information on the substance of interest in the PBO and may refer to typical range found in equivalent TBOs (reference variety) according to proprietary data, data published in the scientific literature, or dataset. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.
- Whether the nutrient or nutrient precursor is decreased or increased.

  Proceed to Section 19.4.

19.4. State whether consumption of the PBO would adversely affect the diet of any population when compared to consumption of a benchmark reference

If no, provide:

- Description of the relevant food or feed characteristics including the main nutrients they provide (for example, protein and/or carbohydrates and/or fatty acids etc).
- The role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed produced from the PBO; details of consumption databases used to conduct the analysis (such as the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020; 2024)).
- Summary of the results of a literature search for any associated health risks.

Proceed to Section 19.5.

If yes, provide:

- Description of the relevant food or feed characteristics including the main nutrients they provide (for example, protein and/or carbohydrates and/or fatty acids etc).
- Brief description of any health risks associated with increased and/or very high levels of the targeted nutrient, if the target nutrient is increased.
- Description of any population that may be vulnerable to undernutrition if the target nutrient is decreased.
- 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.

  This is a substance of concern: further information on this substance will need providing according to Section 26 to support its Tier 2 safety assessment. Proceed to Section 19.5.

19.5. State whether the substance of interest significantly alters bioaccessibility

If no, provide:

- Identification of the substance of interest (as per Section 17.1).
- Statement of confirmation that there is no evidence of established effect on bioaccessibility for this substance in the scientific literature, referencing the databases or scientific literature sources reviewed to reach this conclusion; the choice of any benchmark reference must be scientifically justified according to Sections 17.2, 17.3.

  Proceed to Section 19.6.

If yes, provide:

- Identification of the substance of interest (as per Section 17.1).
- Brief description of the alteration of bioaccessibility with reference to the available literature relating to any affected biological pathways, detailing any effects relevant to bioavailability including factors affecting digestibility and absorption.

  This is a substance of concern: further information on this substance will need providing according to Section 26 to support its Tier 2 safety assessment. Proceed to Section 19.6.

19.6. State whether the substance of interest is an antinutrient or an adjuvant

If no, provide:

- Statement confirming the substance of interest is not a known antinutrient or an adjuvant.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Nutrition Tier 1 safety assessment for this substance.

If yes, provide:

- Statement confirming whether the substance of interest is a known antinutrient or an adjuvant. Include a brief description of the antinutrient or adjuvant and its classification (for example, Tannin, Lectin *etc.*).

  Proceed to 19.7.

19.7. State whether levels or activity of the substance are significantly reduced compared to a benchmark reference

If no, provide:

- Summary of the analysis comparing the levels of the substance in each part from the PBO destined for food or feed use with those found in a reference variety from a species with HSFU, including statistical analysis. This must use proprietary compositional information on the substance of interest in the PBO and may refer to typical range found in equivalent TBOs (reference variety) according to proprietary data, data published in the scientific literature, or dataset. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.
- Clearly state where levels or activity of the substance have increased.

Proceed to 19.8.

If yes, provide:

- Statement of confirmation that levels are below or not significantly different from a benchmark reference. This must describe how levels compare with those found in a reference variety from a species with HSFU, including statistical analysis, using proprietary compositional information on the substance of interest in each part from the PBO destined for food or feed use; this may refer to typical range found in equivalent TBOs. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Nutrition Tier 1 safety assessment for this substance.

19.8. State whether levels are expected to be above acceptable levels in food/feed from the PBO after anticipated processing

In this section, 'acceptable' levels refer to levels/activity equivalent to or below those found in food/feed from a reference variety after the same anticipated processing.

If no, provide:

- Brief description of how antinutrients are removed or inactivated by typical industrial or domestic processing before consumption, together with an identification of the method of reduction (for example, soaking, fermentation, heat treatment, milling, roasting) involved in the removal.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Nutrition Tier 1 safety assessment for this substance.

If yes, provide:

- Brief description of the evaluation of any anticipated industrial or domestic processing showing levels will be above acceptable levels after processing.

  This is a substance of concern: no further information is needed on this substance as part of Tier 1 safety assessment, but further information on this substance will need providing according to Section 26 to support its Tier 2 safety assessment.

19.9. Conclusion to the Nutrition safety assessment

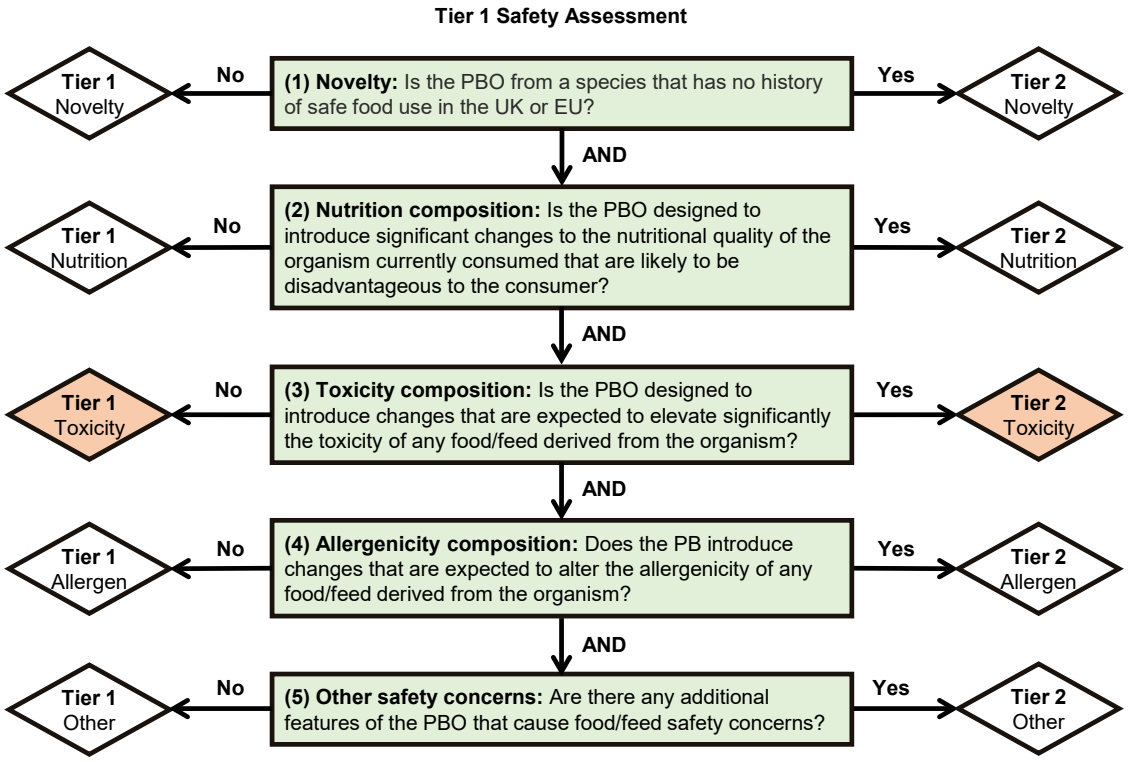
When all identified substances of interest have been assessed and none of them require a Tier 2 safety assessment, provide:

- Statement of confirmation that there is no nutrition concern for the PBO.

When all identified substances of interest have been assessed and any of them require a Tier 2 safety assessment, provide:

- Conclusion that a Tier 2 assessment is needed to review the nutrition of the PBO, identifying the substance(s) of interest resulting in a concern.

20. Information on Toxicity to include in all applications



20.1. State whether any substance(s) of interest were identified in the PBO

If no, provide:

- Statement of confirmation that no substance of interest was identified from considering the phenotype resulting from the genetic change(s), and that as a consequence there is no safety concern over toxicity for the PBO.

  No further information is needed on the Tier 1 toxicity safety assessment.

If yes, follow the instructions in Sections 20.2 to 20.9 **for each identified substance of interest**. The submission should consist of individual summaries of the assessment of each substance of interest, and a final overall conclusion to the Tier 1 toxicity assessment.

20.2. State whether the substance of interest is new to food / feed use

If no, provide:

- Identification of the substance of interest (as per Section 17.1).

- Statement of confirmation that this substance is present in food / feed commonly consumed.

  Proceed to Section 20.3.

If yes, provide:

- Identification of the substance of interest (as per Section 17.1), clearly stating that this substance is not commonly found in the diet, and is a substance of concern which needs further Tier 2 safety assessment.
- Summary of the compositional data on the substance for each part intended for food and feed (as per Section 17.2).
- Identification of the processing method currently used by anticipated processors for the benchmark reference, and how this may impact the substance (removal and/or inhibition and/or alteration); this must use current knowledge of food safety management systems and appropriate supporting evidence (references, test results etc); specify whether the conclusions are evidenced by published literature, or proprietary data; anticipated levels in the food/feed product or range of intended food/feed products should be provided.
- The role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrients they provide (for example, protein and/or carbohydrates and/or fatty acids etc); details of consumption databases used to conduct the analysis (such as the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020), or the EFSA statement on the animal dietary exposure in the risk assessment of contaminants in feed (2024)).

  This is a substance of concern: no further information is needed on this substance as part of Tier 1 safety assessment, but further information on this substance will need providing according to Section 27 to support its Tier 2 safety assessment.

20.3. State whether the substance of interest has established toxicity, including toxicity by threshold

If no, provide:

- Statement of confirmation that there is no evidence of established toxicity of this substance in the scientific literature, referencing the databases or scientific literature sources reviewed to reach this conclusion. Toxicity by threshold must be firmly established in the literature; toxicity based on putative function are not considered evidence of toxicity if not supported by experimental information.

This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Toxicity Tier 1 safety assessment for this substance.

If yes, a summary of the compositional data on the substance for each part intended for food and feed must be used as evidence in the following steps. Proceed to Section 20.4.

20.4. State whether the substance of interest is a protein toxin whose structure was changed in a way anticipated to significantly increase its toxicity

If not applicable, or if no,

Where the substance is not a protein whose encoding sequence has been altered by the genetic change, proceed to Section 20.5.

Where the changes in structure are presumed safe, provide:

- 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);
- Statement of confirmation that the change in structure is presumed safe: this must be supported by a summary of the functional sequence analysis and conclusion on the resulting change in the toxicity of the protein; specify whether the conclusions are evidenced by *in silico* prediction methods (for example, as reviewed by Palazzolo *et al.*, (2020, 2024), published research in peer reviewed journals, or proprietary data, the detail of which does not need to be provided when a Tier 1 safety assessment is sufficient.

  Proceed to Section 20.5.

If yes, provide:

- Brief description of the intended benefits, where relevant.
- 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).
- Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the toxicity of the protein; specify whether the conclusions are evidenced by *in silico* prediction methods (for example, as reviewed by Palazzolo *et al.*, (2020, 2024), published research in peer reviewed

journals, or proprietary data, the detail of which does not need to be provided when a Tier 1 safety assessment is sufficient.

- Identification of the parts of the plant for food/feed use containing the altered protein.

Proceed to Section 20.8.

20.5. State whether the substance of interest is present at biologically relevant ranges

If no, provide:

- Statement of confirmation that levels in the PBO are not expected to be biologically relevant, supported by a summary of the scientific rationale identifying ranges of concentration required to exert toxicity, and referencing the databases or scientific literature sources reviewed to reach this conclusion.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Toxicity Tier 1 safety assessment for this substance.

If yes, provide:

- Brief description of the ranges for which the substance is expected to be biologically relevant, referencing the relevant databases or scientific literature sources.

  Proceed to Section 20.6.

20.6. State whether the PBO is a new dietary source for the substance of interest

If no, provide:

- Statement of confirmation that the substance is produced in the parent organism, referencing the databases or scientific literature sources.

  Proceed to Section 20.7.

If yes, provide:

- Brief description of the mechanism allowing the expression of the substance in the PBO.

  Proceed to Section 20.8.

20.7. State whether the levels of the substance of interest are significantly increased in the consumed parts compared to a benchmark reference

If no, provide:

- Statement of confirmation that levels of the substance have been monitored and comply with existing legal limits, or
- Statement of confirmation that levels are presumed safe according to HSFU/PFC (within the normal range found in equivalent TBOs or other scientifically reasoned reference). This must describe how levels compare with those found in a reference variety from a species with HSFU, including statistical analysis, using proprietary compositional information on the substance of interest in each part from the PBO destined for food or feed use; this may refer to typical range found in equivalent TBOs. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Toxicity Tier 1 safety assessment for this substance.

If yes, provide:

- Summary of the analysis comparing the levels of the substance in each part from the PBO destined for food or feed use with those found in a reference variety from a species with HSFU, including statistical analysis. This must use proprietary compositional information on the substance of interest in the PBO and may refer to typical range found in equivalent TBOs (reference variety) according to proprietary data, data published in the scientific literature, or dataset. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.

  Proceed to Section 20.8.

20.8. State whether the levels of the substance of interest are expected to be above acceptable levels in food/feed from the PBO after anticipated processing

In this section, 'acceptable' levels refer to levels/activity equivalent to or below those found in food/feed from a reference variety after the same anticipated processing.

If no, provide:

- Statement of confirmation that levels of the substance will be reduced to acceptable levels through current standard practices of food safety management. This must identify the processing method currently used by anticipated processors as part of food safety management systems to control the levels / activity of the substance from the benchmark reference (this must use current knowledge of food safety management systems), and evaluate 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.
- Range of levels at which the substance is expected to be biologically active; where possible, anticipated levels in the food/feed product or range of intended food/feed products must be provided.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Toxicity Tier 1 safety assessment for this substance.

If yes, provide:

- Identification of the processing method currently used by anticipated processors as part of food safety management systems to control the levels / activity of the substance from the benchmark reference; 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.
- Range of levels at which the substance is expected to be biologically active; where possible, anticipated levels in the food/feed product or range of intended food/feed products must be provided.

  Proceed to Section 20.9.

20.9. State whether the dietary exposure to the levels of the substance could result in adverse consequences for the consumer

If no, provide:

- The role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrients they provide (for example, protein and/or carbohydrates and/or fatty acids etc); details of consumption databases used to conduct the analysis (such as

the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020), 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; this may refer to datasets such as the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020), the EC Novel Food status catalogue.
- Statement of confirmation that dietary exposure will not result in adverse consequences for the consumer. This must be supported by a summary of the scientific rationale as to why expected levels of substance are not anticipated to result in significantly increased exposure compared to that expected from equivalent TBOs; benchmark references must be justified as described in Sections 17.2 and 17.3; specify whether the conclusions are based on predictive or proprietary quantitative information on the levels in the PBO.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Toxicity Tier 1 safety assessment for this substance.

If yes, provide:

- A brief referenced summary of any health risk associated with increased 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.
- The role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrients they provide (for example, protein and/or carbohydrates and/or fatty acids etc); details of consumption databases used to conduct the analysis (such as the EFSA Comprehensive Food Consumption Database (2018), the Public Health England NDNS dataset (2020), or the EFSA statement on the animal dietary exposure in the risk assessment of contaminants in feed (2024)).

  This is a substance of concern: no further information is needed on this substance as part of Tier 1 safety assessment, but further information on this substance will need providing according to Section 27 to support its Tier 2 safety assessment.

20.10. Conclusion to the Toxicity safety assessment

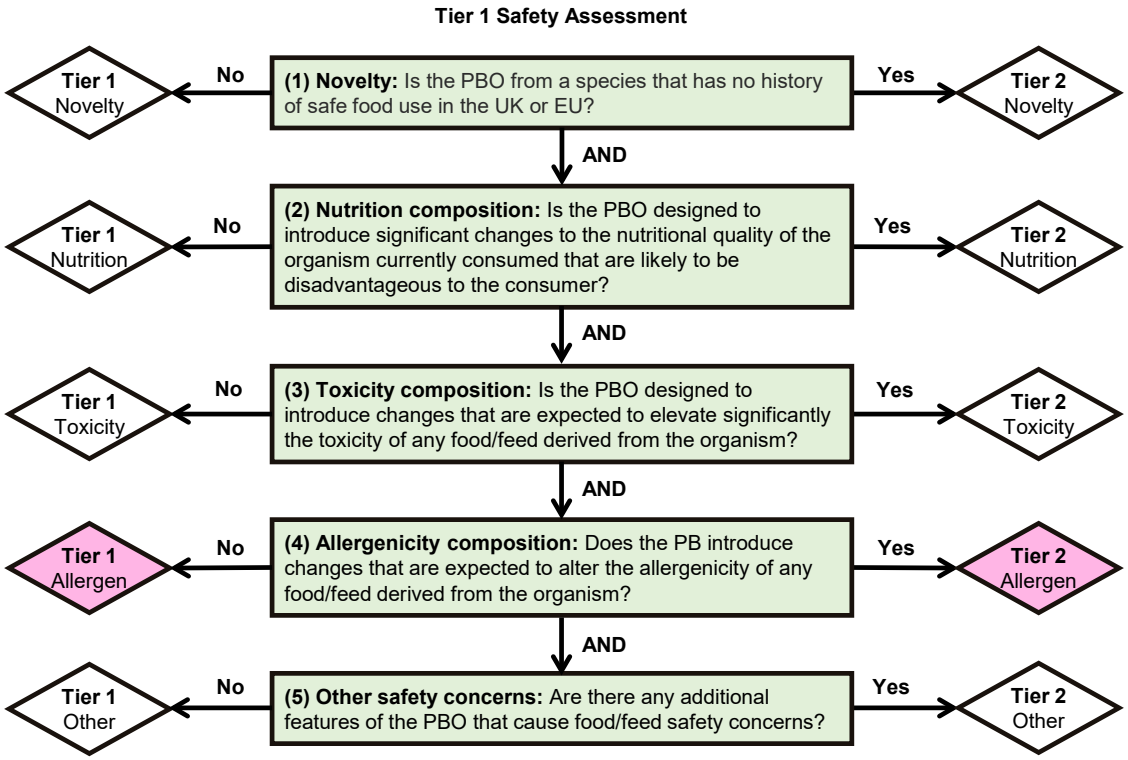
When all identified substances of interest have been assessed and none of them require a Tier 2 safety assessment, provide:

- A statement of confirmation that there is no toxicity concern for the PBO.

When all identified substances of interest have been assessed and any of them require a Tier 2 safety assessment, provide:

- Conclusion that a Tier 2 assessment is needed to review the toxicity of the PBO, identifying the substance(s) of interest resulting in a concern.

21. Information on Allergenicity to include in all applications



21.1. State whether the host plant and/or the cisgene donor is a clinically relevant allergenic organism

If no, provide:

- A statement of confirmation that the host organism/cisgene donor species is not an allergenic organism, and that there is no reason to believe that the allergenicity of the PBO may be changed.

  No further information is needed on the Tier 1 allergenicity safety assessment.

If yes, provide:

- A statement of confirmation acknowledging the host organism and/or cisgene donor has a significant history of inducing allergic responses.

  Proceed to Section 21.2.

21.2. State whether there is a clinically relevant allergen amongst the identified substances of interest

If no, provide:

- A statement of confirmation that there is no evidence in the scientific literature of clinically relevant allergenicity for any of the identified substances of interest.

  No further information is needed on the Tier 1 allergenicity safety assessment.

If yes, provide**:**

- A statement of confirmation that the host organism and/or cisgene donor has a significant history of inducing allergic responses.

  Follow the instructions in Sections 21.3 to 21.6 **for each identified clinically relevant allergen (those linked to the phenotype and potential changed allergenicity)**. The submission should consist of individual summaries of the assessment of each substance of interest, and a final overall conclusion to the Tier 1 toxicity assessment.

21.3. State whether the purpose of the change is to reduce the allergenicity of the PBO to promote consumption (including by an allergic population)

If no, provide:

- Identification of the allergen (as per Section 17.1);
- Statement of confirmation that there is no intention to market the PBO with a claim of reduced allergenicity for the purpose of promoting consumption (including by an allergic population).

  Proceed to Section 21.4.

If yes, provide:

- Identification of the allergen (as per Section 17.1);
- Clear identification of the parts of the plant for food or feed use which are expected to have reduced allergenicity.

Where the decrease in allergenicity in the PBO results form a decrease in levels of the allergen:

- **Compositional data** quantifying the allergenic proteins whose levels are expected to be significantly decreased, compared to a suitable benchmark reference (see Section 17.2).
- Summary of the analysis comparing the levels of the allergen in each part from the PBO destined for food or feed use with those found in a reference variety from a species with HSFU, including statistical analysis. This must use proprietary compositional information on the allergen in the PBO and may refer to typical range found in equivalent TBOs (reference variety) according

to proprietary data, data published in the scientific literature, or dataset. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.

- Reasoned scientific conclusion on the expected effect on the allergenicity of food or feed produced from the PBO. Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

Where the decrease in allergenicity in the PBO results from a change in structure of the allergen:

- Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the allergenic properties of the protein and how it may alter the allergenicity of any food or feed produced from the PBO. For example, this may include a summary of structural modelling in combination with robust prior experience with the crop and the trait, and peer-reviewed scientific literature. Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

This is a trait of concern: no further information is needed on this allergen as part of Tier 1 safety assessment, but further information on the PBO will need providing according to Section 28 to support its Tier 2 safety assessment.

21.4. State whether the structure of the allergen is changed in a way anticipated to significantly alter its allergenic properties

Where the allergen encoding sequence has not been altered by the genetic change, proceed to Section 21.5.

If no, provide:

- Statement of confirmation that the changes in structure are presumed safe, supported by a summary of the functional sequence analysis of the structural changes.
- Whether a **change in structure** could significantly increase allergenicity of a protein and could alter the allergenicity of the produced food must be a scientifically reasoned conclusion; specify whether the conclusions are based on sequence analysis, published research in peer reviewed journals, or proprietary data.

  Proceed to Section 21.5.

If yes, provide:

- Identification of the parts of the plant for food use containing the altered protein.
- Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the allergenicity of the protein.
- Reasoned scientific conclusion for why the alteration to the protein structure is considered significant, and how it may alter allergenicity. For example, this may include a summary of structural modelling in combination with robust prior experience with the crop and the trait, and peer-reviewed scientific literature.
- Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.

Proceed to Section 21.6.

21.5. State whether the levels of the allergen are significantly altered in the consumed parts

If no, provide**:**

- **Compositional data** quantifying the allergenic proteins whose levels are expected to be significantly altered, compared to a suitable benchmark reference (Section 17.2).
- Statement of confirmation that levels are not significantly different from a benchmark reference. This must describe how levels compare with those found in a benchmark reference, including statistical analysis, using proprietary compositional information on the allergen in each part from the PBO destined for food or feed use; this may refer to typical range found in equivalent TBOs. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.
- Statement whether the change in allergen levels is significant and reasoned scientific conclusion on safety regarding allergenicity. Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.
- Brief rationale, with referenced evidence, that levels are presumed safe according to HSFU/PFC.

This ends the Tier 1 safety assessment for this allergen; no further information needs to be provided on Allergenicity Tier 1 safety assessment for this allergen.

If yes, provide:

- Clear identification of the parts of the plant for food or feed use which may contain altered levels.
- **Compositional data** quantifying the allergenic proteins whose levels are expected to be significantly altered, compared to a suitable benchmark reference (see Section 17.2).
- Summary of the analysis comparing the levels of the allergen in each part from the PBO destined for food or feed use with those found in a reference variety from a species with HSFU, including statistical analysis. This must use proprietary compositional information on the allergen in the PBO and may refer to typical range found in equivalent TBOs (reference variety) according to proprietary data, data published in the scientific literature, or dataset. In all cases, the choice of the benchmark reference must be scientifically justified, and compositional information must be provided as described in Sections 17.2 and 17.3, including a scientific justification of the sampling plan.
- Statement whether the change in allergen levels is significant and reasoned scientific conclusion on the possible effect on safety regarding allergenicity. Specify whether the conclusions are evidenced by genetic and/or physiological knowledge, and/or published literature or proprietary data.
- For **cisgenesis** only, 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.

  Proceed to Section 21.6.

21.6. Where allergenicity could be altered, state whether published clinical studies for the same trait in this species has demonstrated unchanged allergenicity

If no, proceed to Section 21.7.

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.

  This ends the Tier 1 safety assessment for this allergen; no further information needs to be provided on Allergenicity Tier 1 safety assessment for this allergen.

21.7. Where allergenicity could be altered, state whether a benchmark reference for the same trait has a history of consumption in the UK or the EU

If no, this is a substance of concern: no further information is needed on this substance as part of Tier 1 safety assessment, but further information on this substance will need providing according to Section 28 to support its Tier 2 safety assessment.

If yes, provide:

- Benchmark references must be justified as described in Section 17.3.
- Scientifically reasoned conclusion on the safety outcome of the PBO based on it exhibiting the same trait resulting from a comparable genetic change.

  This ends the Tier 1 safety assessment for this substance; no further information needs to be provided on Allergenicity Tier 1 safety assessment for this substance.

21.8. Conclusion to the Allergenicity safety assessment

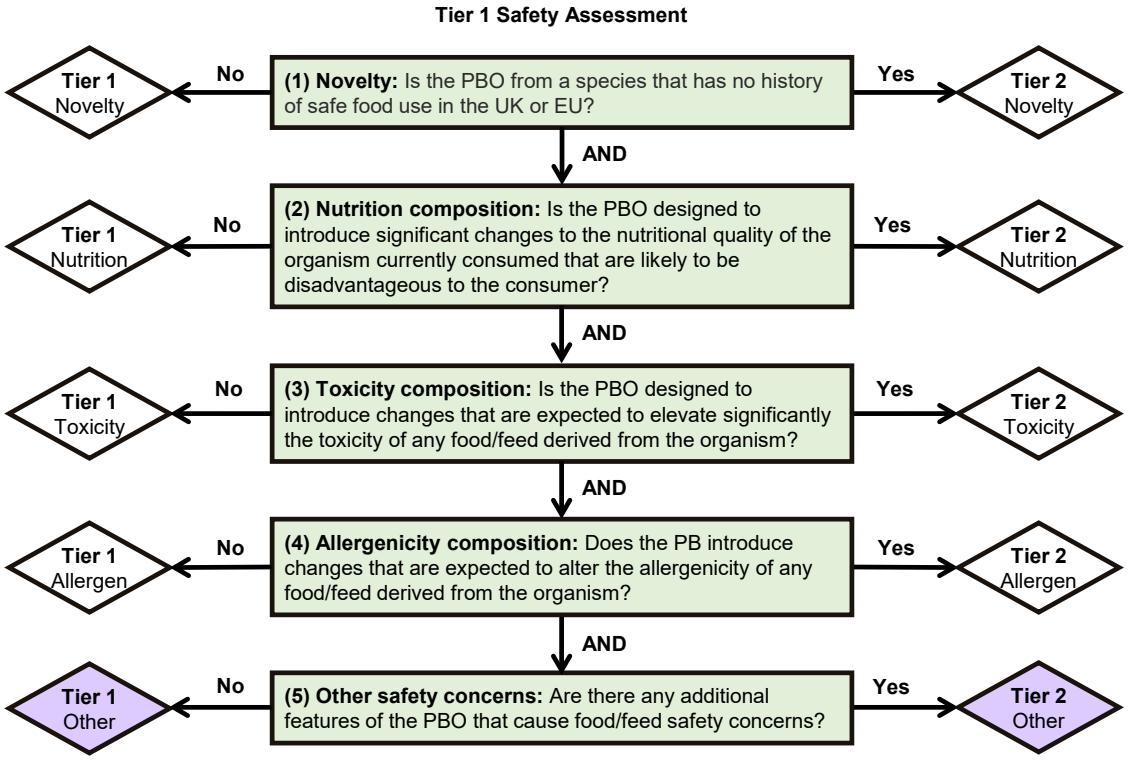
When all identified allergens of interest have been assessed and none of them require a Tier 2 safety assessment, provide:

- A statement of confirmation that there is no potential altered allergenicity concern for the PBO.

When all identified substances of interest have been assessed and any of them require a Tier 2 safety assessment, provide:

- Conclusion that a Tier 2 assessment is needed to review the allergenicity of the PBO, identifying the allergen(s) of interest resulting in a concern.

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



The questions outlined in Sections 15.2.1, 15.2.2, 15.2.3, 15.2.4 and 15.2.5 provide a guide to assessing other safety concerns and identifying what requires a Tier 2 safety assessment (also see Figure 10). 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 foreseeable safety concerns they are aware of.

Where no other safety concern has been identified, provide:

- Statement of confirmation that to the best of the applicant's knowledge, the PBO does not present any other safety concerns.

Where any other safety concern has been identified, provide:

- Conclusion that a Tier 2 assessment is needed, identifying the cause(s) of other safety concern.

23. How to identify additional information required to support Tier 2 safety assessment

Applicants must refer to Part 5 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 5 - Additional information to include in support of a 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.

24. General considerations on the information to include for Tier 2 safety assessment

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 must 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 by the applicant. 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. Changes intended to be beneficial to the consumer may also need to be assessed to ensure that altered exposure in the diet will not be detrimental to any population (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 28.2 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.

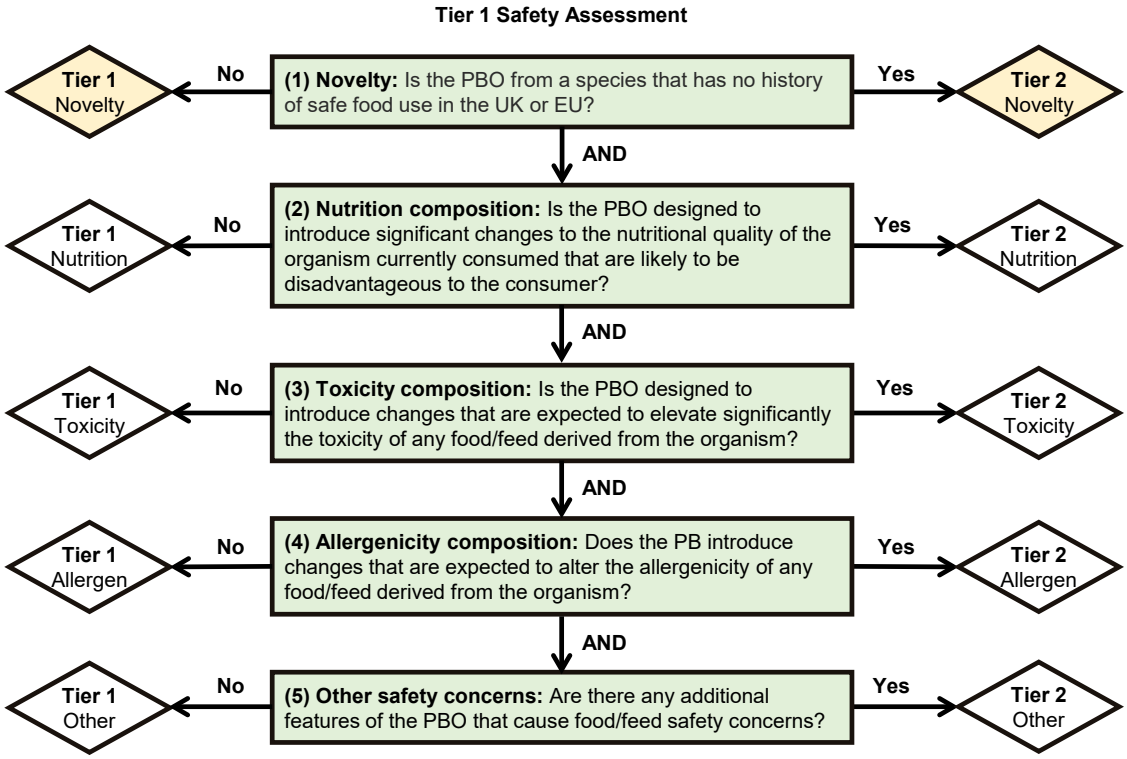
24.1. Compositional information to provide on the PBO for Tier 2 safety assessment

The analytical data for Tier 2 safety assessment must document any substance of interest that raised a concern as identified in Novelty, Nutrition, Toxicity and Allergenicity sections. Where additional compositional data is required (for example to support Tier 2 safety assessment of Novelty or of Nutrition), it is clearly identified in the relevant sections of the guidance. Compositional data should be provided as follows:

- Raw data for a minimum of 5 representative samples of the PBO independently harvested and of its benchmark reference, submitted in the form of a table; the analytical method for chemical analyses and supporting certificates of analysis should be provided.
- When mean is used for comparison, a description of the statistical methods used, and results of the statistical analysis.

Section 9.2 describes the analytical requirements which should be met.

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



See Section 24 for initial requirement for a Tier 2 safety assessment.

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 subjected 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 12, 13 and 14.

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 subjected 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 the scientific requirements for an application for authorisation of a novel food in the context of Regulation 2015/2283 (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.

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 9.2, 9.3).

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, 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 Guidance on the scientific requirements for a notification and application for authorisation of traditional foods from third countries in the context of Regulation 2015/2283 (2024b) and with section 2 of the EFSA guidance on novel foods (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 (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 (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 (2024b) and with section 6 of the EFSA guidance on novel foods (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 benchmark reference (a suitable benchmark reference 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 12.

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 (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 amino acid 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 13.

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 13.2 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 (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 benchmark reference, 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, differences in target animal species 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 (2012). This may include: toxicokinetics (OECD TG 417); genotoxicity (OECD TG471, TG 487, TG 474, TG 488, TG 489, reviewed in EFSA Scientific Opinion (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 14.

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 (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.

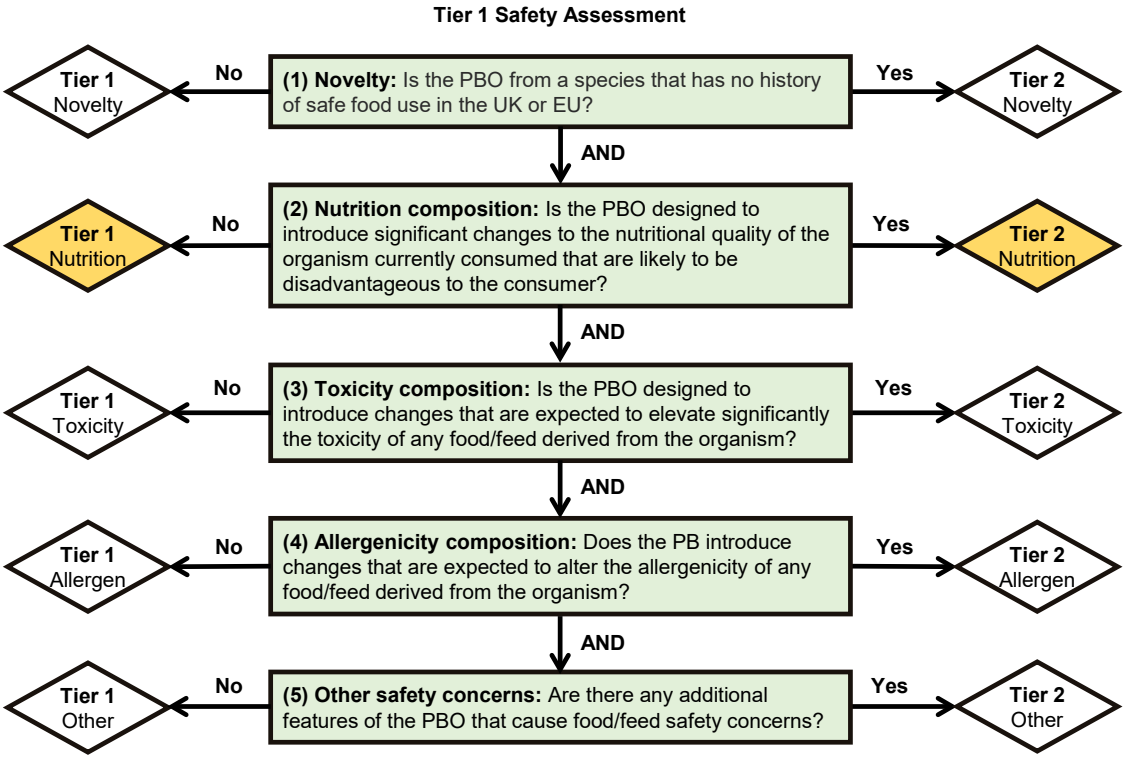
25.12. Concluding remarks on the PBO requiring Tier 2 safety assessment for Novelty

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 a Tier 2 safety assessment. 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 benchmark references, applicants must demonstrate that the nutritional quality is not adversely affected.

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.

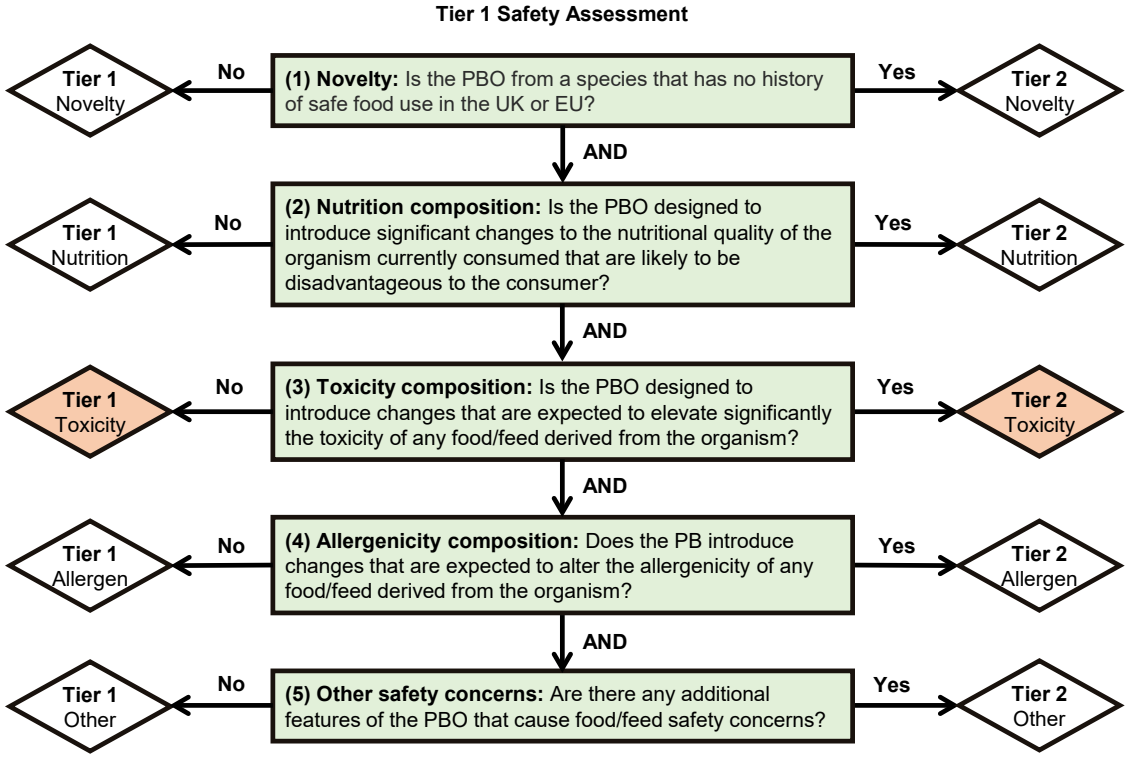
In addition, the following data are requested if **nutrient content** is of concern:

- **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 are known to 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, should be reported.

In addition, the following data are requested if **bioaccessibility** 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.

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



See Section 24 for initial requirement for a Tier 2 safety assessment. 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 Sections 9.2, 9.3).

Provide:

- Qualitative and quantitative data on the levels of substance(s)/protein(s) of possible concern to human health identified in Section 13.2. Data must include the raw data, the mean, range, and error of the levels of the substance(s). Data must be obtained from each part 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 REACH Guidelines (Health and Safety Executive) and FSA UK NAMs Roadmap (Committee on Toxicity, 2024).

27.3. Experimental design, template and benchmark reference 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 benchmark reference, 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, differences in target animal species 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:

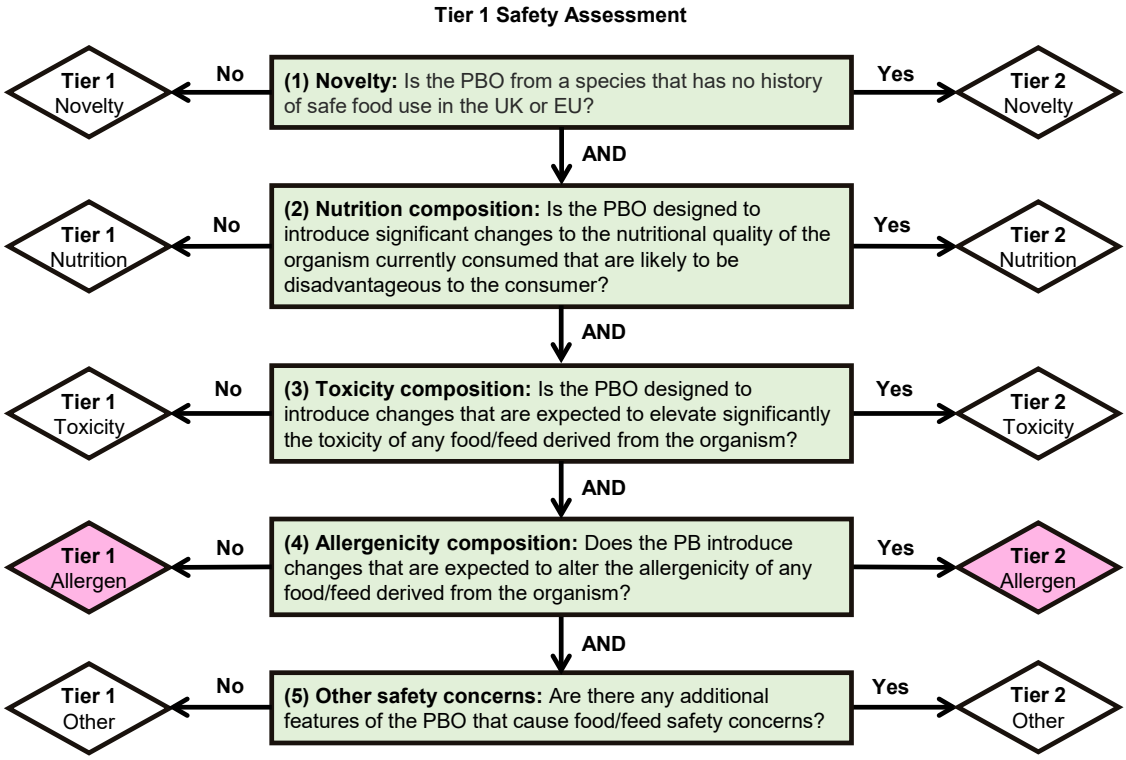
- Brief description of absorption and breakdown as reported in the literature, and of chemical and physicochemical data.
- Brief description of *in vitro* absorption data and *in vitro* comparative gastrointestinal metabolism data (to establish whether the substance or breakdown products are absorbed 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 (2012). This may include: toxicokinetics (OECD TG 417); genotoxicity (OECD TG471, TG 487, TG 474, TG 488, TG 489, reviewed in EFSA Scientific Opinion (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 a Tier 2 safety assessment. 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 a suitable benchmark reference used for food 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.

For every substance of interest resulting in an allergenic concern:

- Identification of the target population.
- Description of the intended use of the final product.
- Description of the final product.

- For each part destined for food or feed use impacted by the genetic change: Raw data for the quantity of the allergen compared to a suitable benchmark reference and a brief explanation for data processing/analysis.

Where the PBO is intended to be **allergen-free**, or to have reduced allergenicity to allow consumption by allergic populations, the initial data submission must include:

- Raw data for the quantity of the allergen compared to a suitable benchmark reference and a brief explanation for data processing/analysis.
- Anticipated daily intake.
- Any pre-existing, published clinical study of the same trait evaluating its potential to elicit a reaction in sensitive people, detailing: the publication reference, the number of participants, the form of the food consumed during the oral challenge, a brief summary of the conclusions on allergenic safety and a scientifically reasoned conclusion on the safety outcome of the PBO based on it exhibiting the same trait as the variety in the study.

Where the genetic change(s) **alters the sequence encoding an allergenic protein**:

- Identification of the target allergen.
- Description of the structural change supported by 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* data or published research in peer reviewed journals.

28.2. 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).

28.3. Experimental design, template and benchmark reference 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 tiered approach will be used to maximise the efficiency of the allergenicity assessments and minimise the use of animals. In this hierarchy (tiers) 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.

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

If the requested scientific evidence in the first tier of assessment described below does not assure allergenic safety, the FSA may request the next tier of assessment is performed until enough evidence has been collected to sufficiently understand safety.

- **Allergenicity-tier 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.
- **Allergenicity-tier 2 -** *In vitro* tests on protein stability and digestibility.
- **Allergenicity-tier 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)).

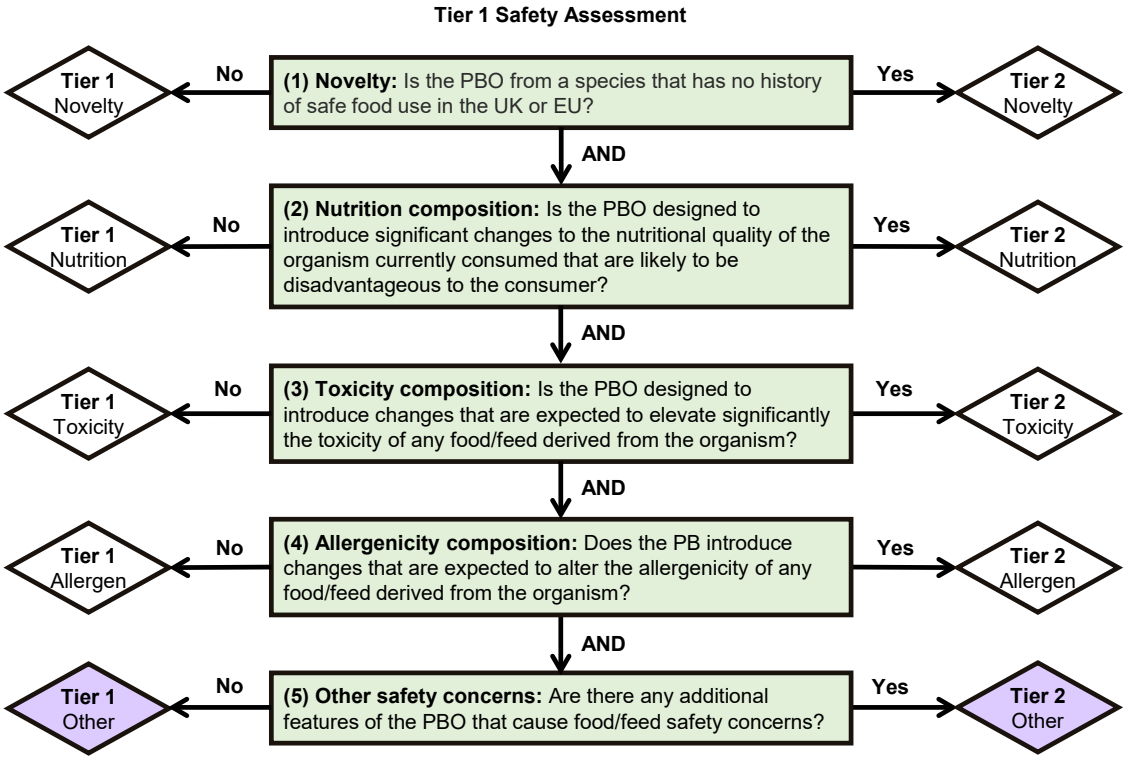
- Evidence of similarity and exposure to the other consumed proteins or species expressing these proteins or similar proteins is needed - reasonable evidence of IgE-mediated oral, respiratory or contact allergy or non-IgE allergy is available on the source of the introduced protein or on the protein 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 benchmark references.
- 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 a Tier 2 safety assessment. All other safety concerns which were identified during the Tier 1 safety assessment should be described.

29.1. Other Safety Concerns associated with required conditions or limitations to the market authorisation

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

- Clear identification of the new condition of use.
- Recommendation of any new risk management measures, if applicable.
- Details, with scientific rationale, of any population groups that should limit or avoid consumption of the PBO, if applicable.
- Details of any historic conditions of use associated with the organism, if applicable.
- For Food use: Description of HSFU.
- For Feed use: Description of PFC.

Where the intention is for a new part (with no HSFU) of the plant to be marketed for food use as a result of the introduced trait, the information required on the new part is in accordance with the EFSA guidance on novel foods (2024c); provide:

- A novelty assessment for the new part of the plant as described in Section 25.

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

- Description of any HSFU, and any other relevant information to support an FSA decision on risk management measures (For example, labelling requirements).

Where the PBO is biofortified, provide**:**

- Brief description of how the increased concentration in the biofortified ingredient is intended to be communicated.
- Recommendation of any risk management measures, if applicable.
- Details, with scientific rationale, of any population groups/animals that should limit or avoid consumption of the PBO, if applicable.

29.2. Other Safety Concerns arising from significantly altered post-harvest handling and processing

Where the genetic change intentionally significantly alters, or could be reasonably anticipated to significantly alter current post-harvest handling and processing of the organism impacting food/feed safety and/or quality**:**

Where the alteration is intentional, provide:

- Detailed description of the process, with a clear identification of the processing step(s) that have been altered, removed or added, including a comparison to existing methods of anticipated major processors and an evaluation of the impact on food safety (including microbiological safety) and nutritional quality, where relevant including any effects on post-harvest management of food safety. Conclusions should be supported by reference to the available scientific literature and compositional data related to the intended change.
- Detailed description of the post-harvest handling step(s) (including storage) that have been altered; evaluation of the impact on food safety and/or nutritional quality (including microbiological safety), where relevant.

Where the alteration is reasonably anticipated, provide:

- Brief description of the changes to post-harvest handling or processing resulting from a similar trait, and of any consequences this had on post-harvest management of food safety.
- Evaluation of the impact on food safety (including microbiological safety) and nutritional quality with reference to the food safety management systems of anticipated major processors, and available scientific literature.

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 (2024c); provide:

- Description of the intended trait and the novel process used to obtain it.
- Provide 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 significantly altered ways of consuming food or feed products from the PBO

Where the alteration is intentional, provide:

- Description of the intended change in consumption, the target population, along with any intended/beneficial use cases.
- Estimation of 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).
- Description of any potential health concerns associated with high levels of consumption by reference to the available scientific literature and considering upper tolerable limits and dietary recommendations.

Where the alteration is reasonably anticipated, provide:

- Brief description of the changes to consumption resulting from a similar trait, and of any consequences this had on post-harvest management of food safety; this may refer to sale data.
- Evaluation of the impact on exposure to substances of concern and on food safety with reference to the available scientific literature.

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

Where any changes in the physical morphology 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 risk management measures that may be necessary.

Where similar combinations of traits in related species are known to present safety concerns, provide:

- Identification of the relevant traits, description of their known hazards, and of any risk management measures that may be necessary.

29.5. Other areas of potential safety concern of which the FSA must be made aware

Where gaps in knowledge or methodological uncertainties hinder accurate Tier 1 safety assessment, provide:

- Description of the gaps in knowledge or methodological uncertainty that hindered the safety assessment.
- Identification of the parts of the Tier 1 safety assessment that were impacted.

Where there is any other scientific reason to believe the product may present safety concerns, based on the available knowledge of the trait(s) and associated phenotype, species and mechanism of action, provide:

- Description of the scientific rationale, clearly identifying the potential safety concern.

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 unless 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 an early draft of 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
COT	Committee on Toxicity (of chemicals in food, consumer products and the environment)
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
GFL	General Food Law
GLP	Good Laboratory Practice
HBGVs	Health-Based Guidance Values
HSFU	History of Safe Food Use
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
RNA	Ribo Nucleic Acid
SAC	Scientific Advisory Committee
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.
Benchmark reference	Benchmark references are used as a comparison point to conclude on significance or safety outcome of a change. They can be used to compare composition, for example compositional data on suitable reference varieties that are proprietary, or from suitable published food composition datasets, or from suitable peer-reviewed scientific literature. They can be used to compare traits. For example, a closely related species (with HSFU and PFC) with the same trait (resulting from a comparable genetic change) and with a

Key words	Definitions
	similar role in the diet can inform conclusions on the safety of food and feed from the PBO.
Bioaccessibility	Bioaccessibility is the proportion of nutrients that are available for absorption.
Biofortification	Biofortification for the purpose of this guidance is the intentional and significant increase in a nutrient in a PBO, as a result of the genetic change rather than by simply adding the nutrient in the way that fortification is typically understood.
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	DNA from the same or a cross-compatible donor species is inserted.
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.
Conditions of use	Specific restrictions or limitations under which food or feed produced from an authorised PBO may be placed on the market in England.
Donor organism	Organism from which an inserted DNA sequence (by cisgenesis or intragenesis) originates.
Edible parts (of the PBO)	Parts of the organism intended for food use, and parts intended for feed use or that may enter the feed chain. The parts intended for feed use may be different from the parts intended for food use. In some instance, the whole organism may be used as feed (silage).
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

Key words	Definitions
	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)). 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.
Intended trait	The intended trait is the characteristic resulting from the genetic change which the precision breeding specifically aimed to develop.
Intragenesis	DNA from the same or a cross-compatible donor species is rearranged before being inserted into the genome of an organism.

Key words	Definitions
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 (Precision Breeding) Regulations (2025).
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.
Nutrient	Nutrients include macro and micronutrients that contribute to the nutritional quality of the edible parts of the PBO.
Nutritional Quality	The nutritional quality of a PBO is defined as the contribution of food or feed consumed from the edible parts of the organism to human and/or animal health, including growth, maintenance and repair.
“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.

Key words	Definitions
Phenotype	The phenotype is the physical or observable expression of traits. The phenotype to assess for PBOs includes the intended trait and any additional associated characteristics reasonably anticipated to result from the genetic change.
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 benchmark reference.
Reasonably anticipated	Predicted or inferred based on well-established current scientific knowledge (for example based on what is known about the function of the gene affected and its product according to published experimental data) or based on existing proprietary data (for example phenotypic observations). Reasonably anticipated effects contribute to the hypothesis-driven identification of concerns.
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 (Precision Breeding) Regulations (2025) have been met.
Regulation 22 application	The application route to be used for a PBO where the criteria in Regulation 22 of the have been met.

Key words	Definitions
Significant (compositional) change	A compositional change is significant if it is outside the ranges found in traditionally bred reference varieties 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 of interest	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. Substances of interest are those substances which levels or activity are predicted to be changed as a result of the genetic change(s), and which are known to have the potential to impact nutrition, toxicity or allergenicity. They must be present in the edible parts of the plant to be relevant to food and feed assessment.
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 or Regulation 22 of the (Precision Breeding) Regulations (2025). 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.

Key words	Definitions
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 have not been met Genetic Technology (Precision Breeding)Regulations (2025). 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.
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.

Annex A – Quick reference guide to the data requirement for all applications

This document is a **quick reference guide to the data required to support** applications seeking authorisation for Precision Bred Organisms (PBOs) for food and feed use; it does not replace the main technical guidance described in Parts 2, 4 and 5 which provides the full detail of the requirement.

Applicants should first identify whether the phenotype introduces any changes in composition relevant to food or feed (Tables 1, 2, 3) before determining whether they are relevant for the quality or safety of food and feed and whether they are significant (Tables 5, 6, 7); this determines the information to be provided in an application.

When substances of interest are identified in Section 8, the questions in Tables 5, 6 and 7 must be answered and evidence provided for each relevant substance.

The sub-questions in each criterion represent the 'key considerations' applicants should take into account and evidence to demonstrate how they reached their conclusions in relation to the criteria set out in paragraphs (1) (b) and (c) of regulation 20 (which are referenced in Schedule 4 (1) (5).

1. Identity (see Section 6)

Table 1. Information to be entered into the text box for "Identity, description of change and safety assessment hypothesis", as relevant. Information already required in the Marketing Notice to the Defra Secretary of State (Defra Marketing Notice, DMN) may be submitted to the FSA where requirements overlap.

Main guidance detailed section	FSA Requirements for all applications
(6.1) Name of the PBO #	Defra unique reference number (URN, Defra registry for authorised PBOs). **For a batch application:** individual identifiers and how many PBOs are included.
(6.2) Taxonomic information * Already required in DMN, Schedule 3 (1)	Scientific (Latin) name including genus, species, according to the international codes of nomenclature.
(6.2) Taxonomic information #	**Where the genetic background may interact with the introduced trait:**

Main guidance detailed section	FSA Requirements for all applications
Additional information required by FSA	• Subspecies or variety; **for a batch application,** subspecies or variety for each included PBO. [or confirmation that the subspecies is not relevant]
(6.3) Purpose of the change * Already required in DMN, Schedule 3 (2)	Brief description of the PBO and the purpose of the altered/introduced trait.
(6.3) Purpose of the change # Additional information required by FSA	**Where relevant -** Further detail on the purpose of the change related to food or feed. [or confirmation that this has no effect on food/feed use]
(6.4) Intended use in food and feed * Already required in DMN, 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).
(6.4) Intended use in food and feed # Additional information required by FSA	Whether the PBO is intended to replace another source of food or feed. **Where only specific parts of the organism are used for food -** Part(s) intended for food use, and whether they are affected by the change introduced by PB. **Where the PBO is used for feed -** Part(s) intended for feed use or that may enter the feed chain, and whether they are affected by the change introduced by PB, specifying when intended use is exclusively in feed. **Where conditions of use that are new to the species are identified for a PBO for food or feed use** - Brief description of new condition(s) of use; brief description of any intended labelling. [or confirmation that there are (as relevant): no intended use in food; no intended use in feed; no new conditions of use]
(6.5) Intended phenotype and rational for targeting the specific genomic region * Already required in DMN, Schedule 2 (5) (e)	• Effect of the introduced change 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;

Main guidance detailed section	FSA Requirements for all applications
	• Intended trait and intended impact of the genetic change on the characteristics (phenotype, including general effects on the physiology) of the organism; • Why the trait was obtained in this particular way, including reasoning for the choice of the target.

***** Information already required in the Marketing Notice to the Defra Secretary of State (Defra Marketing Notice, DMN) as described in Schedule 2 (3), (4) and (5) of the Genetic Technology (Precision Breeding) Regulations (2025); the DMN must be submitted to the FSA with a food and feed marketing authorisation application.

 # Additional information specifically relevant to food/feed use must always be provided for the application to the FSA for a food and feed marketing authorisation.

2. Description of change (see Section 7)

Table 2. Information to be entered into the text box for "Identity, description of change and safety assessment hypothesis", as relevant. Information already required in the Marketing Notice to the Defra Secretary of State (Defra Marketing Notice, DMN) may be submitted to the FSA where requirements overlap.

Main guidance detailed section	FSA Requirements for all applications
(7.1) Targeted sequence changes ***** Already required in DMN, Schedule 2 (5)	• Gene(s) name(s) and alternative name(s) (if in coding sequence); • Primary function or hypothetical function of the coding sequence targeted, or; primary function or hypothetical function (if any) of the non-transcribed sequence targeted; • Gene type, for example, whether it encodes a protein or is transcribed into non-coding RNA.
(7.1) Targeted sequence changes **#** Additional information required by FSA	**Where multiple copies of the target sequence exist in the genome** - whether all copies were altered. **Where level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result:** these must be identified and further examined in (8). [or confirmation that the genetic change instead consists of cisgenesis or intragenesis]
(7.2) Cisgenesis and intragenesis ***** Already required in DMN, Schedule 2 (5)	**For cisgenesis –** detail of the genetic components introduced; how many copies were introduced; donor organism species and/or subspecies. **For intragenesis –** for each genetic component inserted: description of the elements within the inserted DNA fragment; relevant

Main guidance detailed section	FSA Requirements for all applications
	information about the rationale for selecting the specific combination; how many copies were introduced; donor organism species and/or subspecies.
(7.2) Cisgenesis and intragenesis **#** Additional information required by FSA	• Clear identification of any metabolic function new to the plant, and the phenotype they result in. Where reasonably **anticipated**: clear identification of gene(s) normally silent in the plant which are now expressed; clear identification of gene(s) normally expressed in the plant which are now silent or have reduced expression. **Where level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result:** these must be identified and further examined in (8). [or confirmation that no cisgenesis or intragenesis was involved]
(7.3) Location(s) and size(s) of the change(s) / insertion(s) ***** Already required in DMN, Schedule 2 (5)	• Whether it is in the nuclear genome OR in non-nuclear genomes; size of the alteration.
(7.3) Location(s) and size(s) of the change(s) / insertion(s) **#** Additional information required by FSA	**For changes in transcribed regions –** identification of the specific exon or intron targeted, how this affects the amino acid sequence where relevant. **For changes not in transcribed regions –** identification of the closest coding sequences and their functions on both sides, where they are within 1kb of the genetic change. **Where non-random insertion is used:** relevant information about the rationale for selecting the specific site; direction of the insertion relative to the 5' end to 3' end of the DNA strand; description of any identified undesired on-target event resulting from precision breeding and its reasonably anticipated consequences on the compositional phenotype. **Where level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result:** these must be identified and further examined in (8). [or confirmation that no unintended on-target event was identified]
(7.4) Unintended genetic change(s) attributable to the application of	**On identified unintended editing events resulting from precision breeding described in DMN –** information as per 7.1, 7.2, 7.3 and 7.5.

Main guidance detailed section	FSA Requirements for all applications
modern biotechnologies **#**	**Where level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result:** these must be identified and further examined in (8). [or confirmation that no unintended off-target event was identified]
(7.5) Additional anticipated effects from connection to biological pathway **#**	• Identification of any related substance(s) whose levels are indirectly significantly affected by the genetic change; brief description of the mechanisms leading to the changes in levels. **Where level or activity of any substance(s) with potential to impact nutrition, toxicity or allergenicity is expected to change as a result:** these must be identified and further examined in (8). [or statement with rationale as to why no effects on composition are anticipated]

***** Information already required in the Marketing Notice to the Defra Secretary of State (Defra Marketing Notice, DMN) as described in Schedule 2 (3), (4) and (5) of the Genetic Technology (Precision Breeding) Regulations (2025); the DMN must be submitted to the FSA with a food and feed marketing authorisation application.

 # Additional information specifically relevant to food/feed use must always be provided for the application to the FSA for a food and feed marketing authorisation.

3. Substances of interest in edible parts (see Section 8)

Substances of interest are those substances which levels or activity are predicted to be changed as a result of the genetic change(s). They must be present in the edible parts of the plant to be relevant to food and feed assessment.

Table 3. Information to be entered into the text box for "Identity, description of change and safety assessment hypothesis", as relevant.

Main guidance detailed section	FSA Requirements for all applications
(8) Distribution of substance(s) of interest in the edible parts	**For each substance of interest identified in Section 7:** • Description of the edible parts of the organism where the levels or activity of the substance(s) are expected to be changed, taking into account any well-established moonlighting and relevant transportation mechanisms. **Where level or activity of substance(s) of interest are changed in the edible parts –** these must be assessed in Tier 1 for nutrition, toxicity and allergenicity

Main guidance detailed section	FSA Requirements for all applications
	[or, in the Tier 1 safety assessment submission text box, confirmation of the absence of relevant substances of interest]

4. Novelty (see Section 18)

Table 4. Information to be entered into the text box for "Novelty", as relevant, to answer the question whether "the relevant precision bred organism belongs to a species that has a history of safe food use".

Step of Novelty Tier 1 assessment (Section 11)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Novelty Step (1) Is the PBO from a species with **no** History of Safe Food Use (HSFU) in the EU or UK prior to 1997?	No	• Statement concluding on the presence of HSFU, relating to how the PBO is intended to be used as a source of food. • 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. No further information needs providing on Novelty Tier 1 assessment – Complete Tier 1 assessment for Nutrition, Toxicity, Allergenicity and Other Safety Concern.
	Yes	Statement concluding on the absence of HSFU, relating to how the PBO is intended to be used as a source of food. Proceed to Step (**2**) Also complete Tier 1 assessment for: Other Safety Concern for food use; Nutrition, Toxicity, Allergenicity and Other Safety Concern for feed use.
Novelty Step (2) Does the species have a history of safe food use for at least 25 years in at least one third country?	No	The PBO should be subject to further **Tier 2 FSA-led safety assessment**. Information to support the Tier 2 assessment by FSA for **PBOs from an organism with traditional use for food in a third country** (PB-OTUs) will need providing, as described in Sections 25.1, 25.2, 25.3, 25.4 (including additional information for PB-OTUs), 25.5, 25.7, 25.12.

Step of Novelty Tier 1 assessment (Section 11)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	The PBO should be subject to further **Tier 2 FSA-led safety assessment**. Information to support the Tier 2 assessment by FSA for **PBOs from a novel organism for food use** (PB-NvOs) will need providing, as described in Sections 25.1, 25.2, 25.3, 25.4 (including additional information for PB-NvOs), 25.6, 25.7, 25.8, 25.9, 25.10, 25.11, 25.12.

5. Nutrition (see Section 19)

Table 5. Information to be entered into the text box for "Nutrition", as relevant, to answer the question whether the introduced genetic changes are "expected to significantly alter the nutritional quality of the organism as it is being consumed as food or feed at the date of the application in a way that is likely to be disadvantageous to the consumer."

Step of Nutrition Tier 1 assessment (Section 12)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Nutrition Step (1) Did you identify any substance of interest?	No	• Statement of confirmation that no substance of interest was identified (as per Section 8), and that there is no nutrition concern for the PBO. No further information needs providing on Nutrition Tier 1 safety assessment – proceed to the Nutrition Step (**Conclusion**).
	Yes	Proceed to Step (**2**)
Instructions for Steps (2) to (8)	N/A	Step (**2**), and following Step(s) as relevant, **need completing for each individual substance of interest identified in Section 8**. Once all substances of interest have been assessed through the relevant steps, proceed to the **Nutrition Step (Conclusion).**
Nutrition Step (2) Is the substance a nutrient?	No	• Statement of confirmation that the substance is not a nutrient. Proceed to Step (**5**)

Step of Nutrition Tier 1 assessment (Section 12)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	• Statement of confirmation that the substance is a nutrient, and brief description of its role in nutrition (19.2). Proceed to Step (**3**)
Nutrition Step (3) Are levels significantly altered in the consumed parts compared to a benchmark reference?	No	Summary of compositional data must support the conclusions on significance (17.2). • Statement of confirmation that levels are not significantly different from an appropriate and adequately justified benchmark reference, supported by a summary of the analysis of the compositional data (17.2, 17.3, 19.3). Proceed to Step (**5**)
	Yes	Summary of compositional data must support the conclusions on significance (17.2). • Summary of the analysis and conclusion on the significance of the change in levels compared to an appropriate and adequately justified benchmark reference, and identification of the parts of the plant for food or feed use containing increased levels (17.2, 17.3, 19.3). • Whether the nutrient is decreased or increased (19.3). Proceed to Step (**4**)
Nutrition Step (4) Would consumption of the PBO adversely affect the diet of any population when compared to consumption of a benchmark reference?	No	• Brief description of the relevant food or feed characteristics. • Role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO, referencing the consumption databases used to conduct the analysis. • Brief description of any associated health risks, according to the literature. Proceed to Step (**5**)

Step of Nutrition Tier 1 assessment (Section 12)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	This is a substance of concern which must be subject to **further Tier 2 FSA-led safety assessment.** **Where substance levels are increased:** • Brief description of the results of a literature search of any health risks associated with increased and/or very high levels. • Details of any population that may be adversely affected, if any, along with brief description of the analysis leading to this conclusion. **Where substance levels are decreased:** • Details of any population that may be adversely affected, if any, along with brief description of the analysis leading to this conclusion. Proceed to Step (**5**)
Nutrition Step (5) Does the substance significantly alter bioaccessibility?	No	• Identification of the substance of interest (17.1). • Statement of confirmation that there is no evidence in the scientific literature that this substance alters bioaccessibility, including justification of benchmark reference as relevant (17.2, 17.3). Proceed to step (**6**)
	Yes	This is a substance of concern which must be subject to **further Tier 2 FSA-led safety assessment.** • Identification of the substance of interest (17.1). • Brief referenced description of how bioaccessibility is altered, including details on: the affected biological pathways; any effects relevant to bioavailability (19.5). Proceed to Step (**6**)
Nutrition Step (6) Is the substance an antinutrient or an adjuvant?	No	• Statement of confirmation that the substance is not a known antinutrient or adjuvant. This is the end of Tier 1 assessment for this substance - no further information needs providing on Nutrition Tier 1 assessment for this substance.

Step of Nutrition Tier 1 assessment (Section 12)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	• Statement of confirmation whether the substance is an antinutrient or an adjuvant, and brief description of the antinutrient or adjuvant and its classification. Proceed to step (**7**)
Nutrition Step (7) Are levels or activity of the substance significantly reduced compared to a benchmark reference?	No	Summary of compositional data must support the conclusions on significance (17.2). • Summary of the analysis comparing the levels of the substance in each part from the PBO destined for food or feed use with those found in an appropriate and adequately justified benchmark reference from a species with HSFU, including statistical analysis using compositional information on the substance of interest (19.7, 17.2, 17.3). Proceed to step (**8**)
	Yes	Summary of compositional data must support the conclusions on significance (17.2). • Statement of confirmation that levels are below or not significantly different from an appropriate and adequately justified benchmark reference (19.7, 17.2, 17.3). This is the end of Tier 1 assessment for this substance - no further information needs providing on Nutrition Tier 1 assessment for this substance.
Nutrition Step (8) Are levels expected to be above acceptable levels in food/feed from the PBO after anticipated processing?	No	• Brief description of how antinutrients are removed by typical industrial or domestic processing, together with an identification of the processing step involved in the removal (19.8). This is the end of Tier 1 assessment for this substance - no further information needs providing on Nutrition Tier 1 assessment for this substance.
	Yes	This is a substance of concern which must be subject to **further Tier 2 FSA-led safety assessment.** • Brief description of the evaluation of any anticipated industrial or domestic processing

Step of Nutrition Tier 1 assessment (Section 12)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		showing levels will be above acceptable levels after processing. This is the end of Tier 1 assessment for this substance - no further information needs providing on Nutrition Tier 1 assessment for this substance.
Nutrition Step (Conclusion) Does any substance require Tier 2 safety assessment?	No	• Statement of confirmation that there is no nutrition concern over the PBO. No further information needs providing on Nutrition Tier 1 safety assessment
	Yes	Any substance which results in concern in steps (**2**), (**5**) or (**8**) must be subject to further Tier 2 FSA-led safety assessment. • Conclusion that a Tier 2 assessment is needed to review the nutrition of the PBO, identifying the substance(s) of interest resulting in a concern. Further information will need providing for Tier 2 nutrition safety assessment of the substance(s) of concern, as identified in Section 26: **Where nutrient content is of concern**: • The raw data used for the analysis in Step (**3**). • Specify whether the PBO contains a nutrient that is new to the organism. • Proximate analysis (quantification of protein, carbohydrate, fat, vitamin and mineral content). • Any Nutrient-linked phenotypic data. **Where bioaccessibility or antinutrient content is of concern**: • The raw data used for the analysis in Step (**7**). • Any additional data relating to any known antinutritional hazards that may be impacted by the genetic change. • Digestibility data.

6. Toxicity (see Section 20)

Table 6. Information to be entered into free text box for "Toxicity", as relevant, to answer the question whether the introduced genetic changes are "expected to significantly elevate the toxicity of any food or feed produced from the precision bred organism".

Step of Toxicity Tier 1 assessment (Section 13)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Toxicity Step (1) Did you identify any substance of interest?	No	• Statement of confirmation that no substance of interest was identified (as per Section 8), and that there is no toxicity concern for the PBO. No further information needs providing on Toxicity Tier 1 assessment – proceed to the Toxicity Step (**Conclusion**).
	Yes	Proceed to Step (**2**)
Instructions for Steps (2) to (9)	N/A	Step (**2**), and following Step(s) as relevant, **need completing for each individual substance of interest identified in Section 8**. Once all substances of interest have been assessed through the relevant steps, proceed to the **Toxicity Step (Conclusion).**
Toxicity Step (2) Is the substance new to food/feed use?	No	• Identification of the substance of interest (17.1). • Statement of confirmation that the substance is commonly found in the diet. Proceed to Step (**3**)
	Yes	This is a substance of concern which must be subject to **further Tier 2 FSA-led safety assessment.** • Identification of the substance of interest (17.1), clearly stating it is new to the diet. • Processing method used for the production of food or feed from the benchmark reference; evaluation of the efficacy of the methods for removal and/or inhibition; anticipated levels in the food/feed product or range of intended food/feed products (20.2). • Role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrient they provide,

Step of Toxicity Tier 1 assessment (Section 13)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		referencing the consumption databases used to conduct the analysis (20.2). • Summary of the compositional data on the substance for each part intended for food and feed (17.2). This is the end of Tier 1 toxicity assessment for this substance.
Toxicity Step (3) Does the substance have established toxicity, including toxicity by threshold?	No	• Statement of confirmation that there is no evidence of established toxicity of this substance in the scientific literature (20.3). This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
	Yes	Summary of the compositional data on the substance for each part intended for food and feed use must be used as evidence in the following steps. Proceed to Step (**4**)
Toxicity Step (4) For protein toxins: is the structure changed in a way anticipated to significantly increase its toxicity?	N/A, No	Where the substance is not a protein whose encoding sequence has been altered by the genetic change - Proceed to Step (**5**) OR • Statement of confirmation that the changes in structure are presumed safe, supported by a summary of the functional sequence analysis of the structural changes (20.4) - Proceed to Step (**5**)
	Yes	• Brief description of the intended benefits, where relevant. • Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the toxicity of the protein (20.4). • Identification of the parts of the plant for food or feed use containing the altered protein. Proceed to Step (**8**)
Toxicity Step (5)	No	• Statement of confirmation that the levels are not expected to be biologically relevant, supported by a summary of the scientific rationale (20.5).

Step of Toxicity Tier 1 assessment (Section 13)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Is the substance present at biologically relevant ranges?		This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
	Yes	• Brief description of the ranges for which the substance is expected to be biologically relevant (20.5). Proceed to Step (**6**)
Toxicity Step (6) Is the PBO a new dietary source for the substance?	No	• Statement of confirmation that the substance is produced in the parent of the PBO (20.6). Proceed to Step (**7**)
	Yes	• Brief description of the mechanism allowing the expression of the substance in the PBO. Proceed to Step (**8**)
Toxicity Step (7) Are levels significantly increased in the consumed parts compared to a benchmark reference?	No	Summary of compositional data must support the conclusions on significance (17.2). **When legal limits exist -** Statement of confirmation that levels have been monitored and comply with existing legal limits. OR • Statement of confirmation that levels are presumed safe according to HSFU/PFC, supported by a summary of the analysis of the compositional data and a justification of benchmark reference (20.7, 17.2, 17.3). This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
	Yes	Summary of compositional data must support the conclusions on significance (17.2). • Summary of the analysis and conclusion on the significance of the change in levels compared to an appropriate and adequately justified benchmark reference, and identification of the parts of the plant for food or feed use containing increased levels (20.7, 17.2, 17.3). Proceed to Step (**8**)

Step of Toxicity Tier 1 assessment (Section 13)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Toxicity Step (8) Are levels expected to be above acceptable levels in food/feed from the PBO after anticipated processing?	No	• Statement of confirmation that levels will be reduced to acceptable levels through current standard practices of food safety management, supported by a summary of the scientific rational taking into account the processing method used to control the levels / activity of the substance(s) and its efficacy for removal and/or inhibition (20.8). • Range of levels at which the substance is expected to be biologically active, and where possible, anticipated levels in the food/feed product or range of intended food/feed products. This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
	Yes	• Processing method used to control the levels / activity of the substance(s) (20.8). • Evaluation of the efficacy of the methods for removal and/or inhibition (20.8). • Range of levels at which the substance is expected to be biologically active, and where possible, anticipated levels in the food/feed product or range of intended food/feed products. Proceed to Step (**9**)
Toxicity Step (9) Could the dietary exposure result in adverse consequences for the consumer?	No	• Role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrient they provide, referencing the consumption databases used to conduct the analysis (20.9). • Brief evidence of HSFU/PFC for UK or EU populations (20.9). • Statement of confirmation that dietary exposure will not result in adverse consequences for the consumer, supported by a summary of the scientific rationale and conclusions on anticipated exposure, including justification of benchmark reference (20.9, 17.2, 17.3).

Step of Toxicity Tier 1 assessment (Section 13)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
	Yes	This is a substance of concern which must be subject to **further Tier 2 FSA-led safety assessment.** • Brief referenced summary of any health risk associated with increased levels, including details of any populations that may be adversely affected upon exposure (20.9). • Role of the food and feed produced from the PBO in the diet, including: identification of either human or animal population groups that typically consume food or feed from the PBO and identification of the main nutrient they provide, referencing the consumption databases used to conduct the analysis (20.9). This is the end of Tier 1 assessment for this substance - no further information needs providing on Toxicity Tier 1 assessment for this substance.
Toxicity Step (Conclusion) Does any substance require Tier 2 safety assessment?	No	• Statement of confirmation that there is no toxicity concern over the PBO. No further information needs providing on Toxicity Tier 1 assessment.
	Yes	Any substance which results in concern in steps (**2**) and (**9**) must be subject to **further Tier 2 FSA-led safety assessment**. • Conclusion that a Tier 2 assessment is needed to review the toxicity of the PBO, identifying the substance(s) of interest resulting in a concern. Further information will need providing for Tier 2 toxicity assessment, as identified in Section 27: • Qualitative and raw quantitative data used for the analysis in Steps (**2**) and (**7**). • Brief description and justification of toxicological testing strategy, including justifying when toxicological studies are not needed.

Step of Toxicity Tier 1 assessment (Section 13)	Answer	FSA Applications requirements (for further detail, follow the cross-reference links)
		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: • 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.

7. Allergenicity (see Section 21)

Table 7. Information to be entered into the text box for "Allergenicity", as relevant, to answer the question whether the introduced genetic changes are "expected to alter the allergenicity of any food or feed produced from the precision bred organism".

Step of Allergenicity Tier 1 assessment (Section 14)	Answer	FSA Applications requirements (for further detail, follow the cross-reference links)
Allergenicity Step (1) Is the host plant or cisgene donor a clinically relevant allergenic organism?	No	• Statement of confirmation that the host organism/cisgene donor species is not an allergenic organism, and that there is no reason to believe that the allergenicity of the PBO may be changed. This is the end of Tier 1 Allergenicity safety assessment - no further information needs providing on Allergenicity Tier 1 safety assessment.
	Yes	• Statement of confirmation that the host organism and/or cisgene donor has a significant history of inducing allergic responses. Proceed to Step (**2**)
Allergenicity Step (2) Is there a clinically-relevant allergen amongst the identified substances of interest?	No	• Statement of confirmation that there is no evidence in the scientific literature of clinically-relevant allergenicity for any of the substances of interest identified in Section 8. This is the end of Tier 1 Allergenicity safety assessment - no further information needs providing on Allergenicity Tier 1 safety assessment.

Step of Allergenicity Tier 1 assessment (Section 14)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	Proceed to Step (**3**)
Instructions for Steps (3) to (7)	N/A	Step (**3**), and following Step(s) as relevant, **need completing for each individual clinically-relevant allergen of interest identified in Step (2)**. Once all allergens of interest have been assessed through the relevant steps, proceed to the **Allergenicity Step (Conclusion).**
Allergenicity Step (3) Is the purpose of the change to **reduce the allergenicity** of the PBO to promote consumption (including by an allergic population)?	No	• Identification of the allergen (17.1, 21.3). • Statement of confirmation that there is no intention to market the PBO with a claim of reduced allergenicity to promote consumption (including by an allergic population) (21.3). Proceed to Step (**4**)
	Yes	• Identification of the allergen (17.1, 21.3). • Clear identification of the parts of the plant for food or feed use which are expected to have reduced allergenicity. **Where the decrease in allergenicity in the PBO results form a decrease in levels of the allergen**: • Summary of compositional data (17.2). • Summary of the analysis and conclusion on the significance of the change in levels compared to an appropriate and adequately justified benchmark reference, and identification of the parts of the plant for food use containing decreased levels (21.3, 17.2, 17.3). • Scientific reasoning for conclusions on the expected effect on the allergenicity of food or feed produced from the PBO. **Where the decrease in allergenicity in the PBO results form a change in structure of the allergen**: • Identification of the parts of the plant for food or feed use containing the altered protein. • Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the allergenicity of the protein (21.3).

Step of Allergenicity Tier 1 assessment (Section 14)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		• Scientific reasoning for conclusions on the expected effect on the allergenicity of food or feed produced from the PBO. This is trait of concern which is subject to **further Tier 2 FSA-led safety assessment**. Information will need providing for Tier 2 allergenicity safety assessment of this allergen.
Allergenicity Step (4) Is the allergen structure changed in a way anticipated to significantly alter its allergenic properties?	No	Where the allergen encoding sequence has not been altered by the genetic change - Proceed to Step (**5**) OR • Statement of confirmation that the changes in structure are presumed safe, supported by a summary of the functional sequence analysis of the structural changes (21.4). Proceed to Step (**5**)
	Yes	• Identification of the parts of the plant for food use containing the altered protein. • Summary of the functional sequence analysis of the structural changes and conclusions on the impact on the allergenicity of the protein (21.4). Proceed to Step (**6**)
Allergenicity Step (5) Are levels significantly altered in the consumed parts compared to a benchmark reference?	No	• Summary of compositional data must support the conclusions on significance (17.2). • Brief rationale, with referenced evidence, that levels are presumed safe according to HSFU/PFC, supported by a summary of the analysis of the compositional data and a justification of benchmark reference (21.5, 17.2, 17.3). This is the end of Tier 1 Allergenicity safety assessment for this allergen - no further information needs providing on Allergenicity Tier 1 safety assessment for this allergen.
	Yes	• Identification of the parts of the plant for food use containing the altered levels. • Summary of compositional data must support the conclusions on significance (17.2).

Step of Allergenicity Tier 1 assessment (Section 14)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		• Summary of the analysis and conclusion on the significance of the change in levels compared to an appropriate and adequately justified benchmark reference, and identification of the parts of the plant for food use containing increased or decreased levels (21.5, 17.2, 17.3). Proceed to Step (**6**)
Allergenicity Step (6) Have published clinical studies for the same trait in this species demonstrated unchanged allergenicity?	No	Proceed to Step (**7**)
	Yes	• Brief description of the published study, including: reference; number of participants; form of the food consumed during the oral challenge; conclusions on allergenic safety. • Scientific reasoning for conclusions on the safety outcome of the PBO based on it exhibiting the same trait as the variety in the study. This is the end of Tier 1 Allergenicity safety assessment for this allergen - no further information needs providing on Allergenicity Tier 1 safety assessment for this allergen.
Allergenicity Step (7) Does a benchmark reference for the same trait belonging to the same species have a history of consumption in the UK or the EU?	No	This is an allergen of concern which is subject to **further Tier 2 FSA-led safety assessment**. Information will need providing for Tier 2 allergenicity safety assessment of this allergen.
	Yes	• Justification for the choice of the benchmark reference (17.3). • Scientific reasoning for conclusions on the safety outcome of the PBO based on it exhibiting the same trait resulting from a comparable genetic change. This is the end of Tier 1 Allergenicity safety assessment for this allergen - no further information needs providing on Allergenicity Tier 1 safety assessment for this allergen.
Allergenicity Step (Conclusion) Does any substance require Tier 2 safety assessment?	No	• Statement of confirmation that there is no altered allergenicity concern over the PBO. No further information needs providing on Allergenicity Tier 1 safety assessment

Step of Allergenicity Tier 1 assessment (Section 14)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
	Yes	Any allergen or trait which results in concern must be subject to **further Tier 2 FSA-led safety assessment**. • Conclusion that a Tier 2 assessment is needed to review the allergenicity of the PBO, identifying the substance(s) of interest resulting in a concern. Further information will need providing for Tier 2 allergenicity assessment, as identified in Section 28: **For each substance of interest resulting in an allergenic concern:** • Identification of the target population. • Description of the intended use of the final product. • Description of the final product. • Raw data used for the analysis in Step (**4**). **Where the genetic change(s) alters the levels of an allergenic protein**: • Anticipated daily intake. **Where the genetic change(s) alters the sequence encoding an allergenic protein**: • Identification of the target allergen. • Description of the structural change, supported by 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. • Scientifically reasoned conclusion on the resulting change in the allergenicity of the protein. **Where the PBO is intended to have reduced allergenicity to allow consumption by allergic populations:** • Raw data used for the analysis in Step (**3**). • Anticipated daily intake. • Brief description of any published clinical study, of the same trait evaluating its potential to elicit a reaction in sensitive people including: reference; number of participants; form of the food

Step of Allergenicity Tier 1 assessment (Section 14)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		consumed during the oral challenge; conclusions on allergenic safety.

8. Other safety concerns (see Section 22)

Table 8. Information to be entered into the text box for "Other Safety Concern", as relevant, to answer the question whether the introduced genetic changes are "expected to introduce any additional features that may affect the safety of any food or feed produced from the PBO".

Step of Other Safety Concern Tier 1 assessment (Section 15)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
Other Safety Concern Step (1) Does the intended use of the PBO require a market authorisation to be subject to any conditions or limitations?	No	Proceed to Step (**2**)
	Yes	A Tier 2 safety assessment is required, the following information will need providing, as relevant: **Where a restriction of use should apply to the PBO:** • Description of the new condition of use. • Recommended new risk management measure, if applicable. • Description, with scientific rationale, of any population that should limit or avoid consumption of the PBO, if applicable. • Description of any historic conditions of use associated with the organism, if applicable. • Description of HSFU and PFC. **Where new parts of the plant are used in food:** • Information for a novelty assessment for the new part use in food (25). **Where the PBO is for feed use only** • Description of any HSFU, and any other relevant information to support the determination of appropriate management measures. **Where a restriction of use should apply to the PBO:**

Step of Other Safety Concern Tier 1 assessment (Section 15)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		• Brief description of how the increased concentration in the biofortified ingredient is intended to be communicated. • Recommended risk management measure, if applicable. • Description, with scientific rationale, of any population that should avoid limit or avoid consumption of the PBO, if applicable. Proceed to Step (**2**)
Other Safety Concern Step (2) Are there any other safety concerns arising from significantly altered post-harvest handling and processing?	No	Proceed to Step (**3**)
	Yes	A Tier 2 safety assessment is required, the following information will need providing, as relevant: **Where alteration in processing or handling is intended:** • Detailed description of the process, including: clear identification of the altered processing step(s); comparison to existing industry methods; evaluation of the impact on food safety and nutritional quality. • Detailed description of the altered handling step(s), including: evaluation of the impact on food safety and nutritional quality. **Where alteration in processing or handling is reasonably anticipated:** • Brief description of the changes to processing or handling resulting from a similar trait, and of any consequences this had on post-harvest management of food safety. • Evaluation of the impact on food safety and nutritional quality. **Where a novel process is intended to be used in conjugation with the genetic change to produce an intended compositional or structural trait with a food:** • Description of the intended trait and the novel process used to obtain it.

Step of Other Safety Concern Tier 1 assessment (Section 15)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		• Details of food safety management systems that will be used, identifying: critical control points; verification procedure; analytical methods. • Evaluation of the impact on food/feed safety and nutritional quality. Proceed to Step (**3**)
Other Safety Concern Step (3) Is the phenotype reasonably anticipated to significantly alter how food or feed products from the PBO will be consumed?	No	Proceed to Step (**4**)
	Yes	A Tier 2 safety assessment is required, the following information will need providing, as relevant: **Where the change in consumption is intended:** • Description of the intended change in consumption, including target population and any intended/beneficial use cases. • Estimation of the anticipated change in consumption by reference to appropriate consumption databases. • Description of any potential health concerns associated with high levels of consumption considering upper tolerable limits and dietary recommendations. **Where a change in consumption is reasonably anticipated:** • Brief description of the changes to consumption resulting from a similar trait, and of any consequences this had on post-harvest management of food safety. • Evaluation of the impact on exposure to substances of concern and on food safety. Proceed to Step (**4**)
Other Safety Concern Step (4) Are there any other safety concerns arising from traits that are new to the species?	No	Proceed to Step (**5**)
	Yes	A Tier 2 safety assessment is required, the following information will need providing, as relevant: **Where there are changes in the physical morphology that may pose mechanical hazard:** • Description of the change in morphology and the way in which the consumer could be

Step of Other Safety Concern Tier 1 assessment (Section 15)	**Answer**	**FSA Applications requirements** (for further detail, follow the cross-reference links)
		harmed, and of any risk management measures that may be necessary. **Where similar combinations of traits in related species are known to be harmful:** • Identification of the relevant traits, description of their known hazards, and of any risk management that may be necessary. Proceed to Step (**5**)
Other Safety Concern Step (5) Are there any other areas of potential safety concern of which the FSA must be made aware?	No	Proceed to Other Safety Concern Step (**Conclusion**)
	Yes	A Tier 2 safety assessment is required, the following information will need providing, as relevant: **Where there are significant gaps in knowledge or methodological uncertainties:** • Description of the gaps in knowledge or methodological uncertainties that hindered the safety assessment. • Identification of which parts of the Tier 1 safety assessment were impacted. **Where there are other well established scientific reasons to believe there may be safety concerns:** • Description of the scientific rationale, clearly identifying the potential safety concerns. Proceed to Other Safety Concern Step (**Conclusion**)
Other Safety Concern Step (Conclusion) Did you answer 'Yes' in any of the steps?	No	• Statement of confirmation that, to the best of the applicant's knowledge, there is no other safety concern over the PBO. No further information needs providing on other safety concern Tier 1 safety assessment.
	Yes	The information provided on the other safety concerns identified will be reviewed as part of a Tier 2 FSA-led safety assessment.

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