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Allergenicity assessment of new or modified protein-containing food sources and ingredients

R.W.R. Crevel [a], K. Verhoeckx [b], K.L. Bøgh [c], N. Buck [d], A. Chentouf [e], S. Flanagan [f], M. Galano [g], J.A. Garthoff [h], S. Hazebrouck [i], R. Yarham [j], G. Borja [k,*], G. Houben [l,m]

[a] René Crevel Consulting Ltd, Suite A, 82 James Carter Road, Mildenhall, IP28 7HP, UK
[b] University Medical Center Utrecht, Department of Dermatology and Allergology, Utrecht, the Netherlands
[c] National Food Institute, Technical University of Denmark, 2800, Kgs. Lyngby, Denmark
[d] General Mills, Inc. Av. Reverdil 14, 1260 Nyon, Switzerland
[e] Roquette Frères, Toxicology & Safety Unit -Nutrition & Health R&D, 1, rue de la Haute Loge, 62136, Lestrem, France
[f] Mondelēz International, UK
[g] dsm-firmenich, Delft, the Netherlands
[h] Danone Food Safety Center, Danone Nutricia Research, the Netherlands
[i] Université Paris Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, Gif-sur-Yvette, France
[j] InBio, UK
[k] ILSI Europe, Belgium
[l] The Netherlands Organisation for Applied Scientific Research TNO, the Netherlands
[m] University Medical Center Utrecht, the Netherlands

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ABSTRACT

The growing world population, changing dietary habits, and increasing pressure on agricultural resources are drivers for the development of novel foods (including new protein sources as well as existing protein sources that are produced or used in an alternative way or in a different concentration). These changes, coupled with consumer inclination to adopt new dietary trends, may heighten the intake of unfamiliar proteins, or escalate consumption of specific ones, potentially amplifying the prevalence of known and undiscovered food allergies. Assessing the allergenicity of novel or modified protein-based foods encounters several challenges, including uncertainty surrounding acceptable risks and assessment criteria for determining safety. Moreover, the available methodological tools for gathering supportive data exhibit significant gaps. This paper synthesises these challenges, addressing the varied interpretations of "safe" across jurisdictions and societal attitudes towards allergenic risk. It proposes a comprehensive two-part framework for allergenicity assessment: the first part emphasises systematic consideration of knowledge and data requirements, while the second part proposes the application of a generic assessment approach, integrating a Threshold of Allergological Concern. This combined framework highlights areas that require attention to bridge knowledge and data gaps, and it delineates research priorities for its development and implementation.

1. Introduction

Sustainably providing sufficient dietary protein to a growing world population constitutes a major challenge, as does catering for the demand for truly innovative food products with desirable health and environmental attributes. It requires introducing new food sources or rethinking the use of existing sources without compromising food safety. Food protein sources novel to European populations, such as insects, exotic fruits and vegetables, and algal proteins have already been developed and marketed. Many food manufacturers are also looking to expand their product portfolios into health and wellbeing platforms. Several are investigating plant proteins as alternatives to meat and dairy proteins, seeking both a health benefit and a lower environmental and climate impact. Others show an interest in alternative ingredients such as novel or ancient grains like buckwheat and millet or alternative sources of dietary fibre, which could expose the population to new, or rarely used dietary proteins. Changes in available foods (e.g. the use of protein isolates) and a consumer desire to eat them may lead to intake of new proteins or significant increases in the intake of specific existing

* Corresponding author. ILSI Europe AISBL, Avenue E. Mounier 83, Box 6, BE-1200, Brussels, Belgium.
E-mail address: publications@ilsieurope.be (G. Borja).

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Abbreviations

AOP	Adverse Outcome Pathway
COST	European Cooperation in Science and Technology
ED	Eliciting dose
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organisation
GM	Genetically Modified
HRIPT	Human Repeated Insult Patch Test
ILSI	International Life Sciences Institute
ImpARAS	Improved Allergenicity Risk Assessment Strategy
ISP	Ice Structuring Protein
MIE	Molecular Initiating Event
NDA	Nutrition, Novel Foods and Food Allergens
NF	Novel Food
TAC	Threshold of Allergological Concern
US	United States
WHO	World Health Organisation

Table 1
Examples of foods and ingredients and their corresponding novel derivatives.

Food/Ingredient	Derivative
Mung beans	Mung bean protein isolate/concentrate
Wheat	Deamidated gliadin
Canola/rapeseed	Protein isolate
Linseed	Flour
Sunflower	Protein isolate
Rice	Protein isolate
Microalgae	Protein
Mealworms	Whole insect, including protein, fibre, micronutrients
Fish (sardines)	Fish peptides from *Sardinops sagax*
Potato	Potato proteins (coagulated) and hydrolysates thereof
Microfungus	Mycoprotein (*Fusarium venenatum*)
Seaweed	Fermented powder

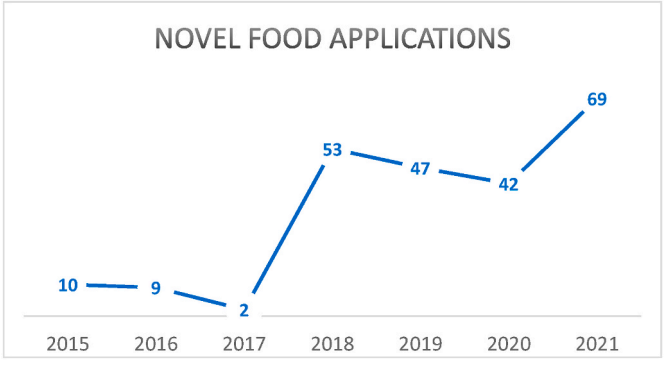

Fig. 1. Number of requests for a scientific opinion of EFSA for a Novel food application. (Based on information available in archive EFSA Register of Questions and Open EFSA Portal (https://open.efsa.europa.eu/questions).

food proteins. Allergenicity is recognised in food safety assessment guidance (EFSA NDA Panel, 2016) as a major hazard to be considered in the context of sources or ingredients based on or containing proteins. To date, when conducted, allergenicity assessment for such new protein sources relies on the Genetically Modified (GM) protein assessment model, [e.g. Codex Alimentarius (CommissionCodex Alimentarius, 2003)] which clearly needs to be updated (Mullins et al., 2022) and is not underpinned by clear and consistent criteria as to what should be considered as acceptable in the food chain.

The definition of new foods can be further extended to cover "existing" foods or food groups (including known allergenic foods) that have been produced by new/novel technologies or where changes have occurred in comparison to foods available in the traditional background diet, including: modification to the protein(s) and/or their structure, a change to the protein concentration or profile (e.g. protein concentrates/isolates) in the foodstuff, a change resulting in increased presence within the diet, or other factors which could conceivably result in a different safety profile, including a modified allergenicity. Table 1 presents a non-exhaustive list of new foods and modifications to existing foods that may lead to changes in exposure to, or health effects from specific proteins within a consuming population.

Perusal of the European Food Safety Authority (EFSA) Register of Questions (https://open.efsa.europa.eu/) and the number of novel food dossiers sent to EFSA illustrates the magnitude of the challenge of meeting the need for safety assessment, including that of allergenicity (Fig. 1).

Assessing the allergenicity of new or modified protein-containing food sources or ingredients raises several issues that underlie the ability to perform an assessment of safety. These include, among others, lack of clarity about what risk(s) is (are) tolerable, as well as the assessment criteria to decide what is considered as acceptable or "safe". Furthermore, the methodological tools available to generate the data required to support any assessment criteria suffer from many gaps, as discussed in several publications (Bogh et al., 2016; Lozano-Ojalvo et al., 2019; Mazzucchelli et al., 2018; Remington et al., 2018; Verhoeckx et al., 2020).

This paper briefly reviews these issues and discusses the interpretation of "safe" in different jurisdictions. Further, it describes the societal acceptability and technological feasibility of testing for parameters and criteria for risk management decision-making. Finally, it proposes a framework for allergenicity assessment which stems from the premise that the risk management question should drive the approach to assessment, including the formulation of the experimental approach.

2. Allergenicity assessment of new or modified protein-containing foods and ingredients: the issues

As already mentioned, the sources and ingredients of interest encompass new uses of existing sources (largely plants), but beyond those and potentially more challenging are totally new sources of proteins to the consumer population in question (e.g. insects, algae, fungi, etc.), which may in some situations be intended as a replacement of existing protein sources. While the substances in question include those defined as novel foods in European Union (EU) legislation 2015/2283/EU (Regulation, 2015a), they extend beyond those to foods or ingredients that would not necessarily require such assessment in the EU, as discussed in a later section. In this document the term "Novel Food" (NF) refers specifically to those defined in EU legislation 2015/2283/EU (Regulation, 2015a). Proteins (and therefore foods and ingredients containing proteins) are recognised as allergenic entities capable of inducing IgE production and an IgE-mediated immune response. In a public health context this particular risk therefore needs to be managed, which requires assessment and safety assurance. The current risk assessment paradigm for any protein source derives from the GM food model:

One or more well-characterised proteins are introduced into an organism. These individual proteins themselves are assessed, as are any unintended effects, such as altered expression of endogenous allergens.

Based on limited evidence to date, this approach seems to have worked well in protecting consumers against allergy to the introduced proteins or an enhanced prevalence of allergy to endogenous allergens, based on an absence of reported effects. However, the situation for new or modified protein-containing food sources and ingredients differs significantly from that around which the GM model evolved:

- The proteins of interest are intended to be consumed in nutritionally significant quantities, unlike proteins engineered into GM crops or even those produced by recombinant DNA techniques for use in food for technical purposes (e.g. ice structuring protein, - ISP).
- The proteins of interest may be presented in diverse ways to those in which they are normally consumed in foods. For instance, they may be used as protein isolates, concentrates or derivatives, containing the constituent proteins in different proportions.
- The proteins of interest may also be modified through processing compared to how they have been traditionally found in foods.
- The proteins can be used in a different matrix to that in which they have been consumed as traditional foods. This matrix may contain other constituents possibly capable of affecting the development of allergy to the proteins, for example saponins.
- The sources or ingredients can contain a multiplicity of proteins that require assessment, rather than a (usually) small number of well-characterised proteins of interest as in the GM case. At best, only those proteins which form a large proportion of the ingredient are likely to be characterised. For some true novel food sources, the proteome has not even been identified and even characterisation of the proteins forming a considerable proportion may be difficult.
- Proteins can also sensitise through routes of exposure (dermal or respiratory) other than the one through which they are normally consumed, although assessment of such routes is not within the scope of this review.

All these considerations would constitute in themselves significant challenges to the assessment of potential allergenicity. However, the situation is made more difficult by the lack of good predictive methods for establishing allergenicity either in the context of sensitisation or elicitation. Together with the lack of clarity over criteria defining the protection goal (i.e., what is "safe"?), the foregoing considerations emphasise that the GM model of risk assessment cannot fulfil the expectations placed on it in this context. Due to these uncertainties, innovation may be hindered, and assurance of safety may be compromised or incomplete.

Current practice in allergenicity risk assessment reflects the uncertainty prevailing in the absence of specific guidance on assessing the allergenicity of new or modified proteins in the diet from non-transgenic sources. Thus, approaches used to date have been diverse, ranging from application of the full panoply of tests specified for transgenic proteins to provision of limited evidence of non-allergenicity from history of (safe) use. Indeed, in some instances, conclusions have been reached without such evidence being presented (Remington et al., 2018). In the EU, legislation has come into force (2015/2283/EU, 2015) specifying the pre-market approval requirements of NF and guidance on requirements has been developed (EFSA NDA Panel, 2016; Dominique et al., 2021) and continues to be developed. However, this guidance does not provide solutions to all issues listed above, while there are many potential modifications to dietary intake of proteins that do not fall under the jurisdiction of this regulation, such as new uses for well-established protein sources. In other regions there is less regulatory oversight specific to NF, but often existing food safety evaluation approaches have been used on a case-by-case basis.

As currently formulated, allergenicity assessment of proteins that are new to the diet or presented in new ways within the diet, fall short on two major counts (Mullins et al., 2022; Verhoeckx et al., 2020):

- *Testing approach and methodology.* While some tests like bioinformatics approaches perform well for assessing cross-reaction of single proteins with existing known allergenic proteins, no current test or combination of tests can yet provide with sufficient certainty information on allergenicity of novel foods containing many proteins. As such there is no experimental data that can be used as the basis for a pre-market quantitative risk assessment, which would enable an understanding of the scale of risk presented by individual modified proteins or risk ranking between proteins, let alone mixtures of protein, as found in foods. Furthermore, no tests exist which could be readily used to screen out new or modified candidate proteins at an early stage of development. Approaches and methodology are even less developed for assessing the allergenicity of the proteins in whole matrices. Development of such tests has also been hindered by the lack of (a) validated reference set(s) of proteins covering a range of allergenic potencies.
- *Regulatory environment.* The criteria on which regulatory decisions are made remain diverse and insufficiently clear, increasing the (business/commercial) risks associated with innovation in this area and thereby potentially reducing development of new food products. This exemplifies the importance of guidance that can be used by food business operators and is recognised by authoritative bodies.

A "level playing field" with well-defined safety requirements regarding allergenicity would support innovation and contribute to a more sustainable food supply.

We propose an approach first developed by the European Cooperation in Science and Technology (COST) Action on Improved Allergenicity Risk Assessment Strategy for new food proteins (ImpARAS; described in some detail in Section 4) whereby the starting point for safety assurance in respect of allergenicity rests with consideration of the health protection goals targeted (Houben et al., 2019). This should form the basis of a strategy for assessing the risk posed by new or modified protein-containing food sources and ingredients. It aims to integrate the risk assessment approach with the risk management objectives via the choice of testing approach and methodologies that provide parameters needed for the assessment, and appropriate assessment criteria. In this context, the risk management objective could be considered as the overarching goal to guide the development of an appropriate assessment approach including suitable hypothesis-driven studies.

3. What is "safe"?

Consumers generally assume that a food offered for sale is safe for them in the absence of contrary indications (e.g. labelling of specific allergenic ingredients posing a risk for people with food allergies), and that safety is assured by the diligence of the food business operator working within a framework of legal requirements. The concept of "safe" implies that the food or its components will not harm consumers when eaten as part of their diet over an indefinite period in quantities that are generally considered reasonable or according to intended use instructions and when stored and prepared in an appropriate manner. Closer examination of the concept of safety in relation to food reveals its complexity insofar as it is not a single, unique characteristic that can be measured, but rather a multidimensional property. A report from the United States (US) National Academy of Sciences (Council, 1998) expresses this rather well: "*Food safety is not limited to concerns related to foodborne pathogens, toxicity of chemical substances, or physical hazards, but may also include issues such as nutrition, food quality, labelling, and education.*"

The Codex Alimentarius defines food safety as "*assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use*" (CAC/RCP,).

In the EU, Article 14 of the General Food Law (Regulation, 2015b) stipulates that "*food shall not be placed on the market if it is injurious to human health or unfit for human consumption*". It clarifies that "*injurious to human health*" should be considered in relation to both the long term and the short-term effects, including those on future generations. It further stipulates that "*in determining whether any food is unsafe regard shall be had … (b) to the information provided to the consumer including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods*". It also stipulates that "*in determining whether any*

food is injurious to health, regard shall be had: … (c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers" (e.g. free from a specific allergen). Importantly, scientific evaluations of foods that have undergone NF evaluation in the EU (e.g. rapeseed protein concentrate (Turck et al., 2023) and mealworm (EFSA Panel on Nutrition, Novel Foods, Food, Allergens et al., 2021a) and mung bean protein (EFSA Panel on Nutrition, Novel Foods, Food, Allergens et al., 2021b) show that allergenicity, either as the ability to sensitise or elicit reactions, does not as such make a NF unsafe as long as the information provided to the consumer is appropriate and/or the risks are considered minimal.

The Food Standard Code developed by Food Standards Australia-New Zealand embodies a similar concept (Food Standard Code, 2022):

For the purposes of the Food Safety Standards, food is not safe if it would be likely to cause physical harm to a person who might later consume it, assuming it was:

(a) after that time and before being consumed by the person, properly subjected to all processes (if any) that are relevant to its reasonable intended use; and
(b) consumed by the person according to its reasonable intended use.

It also illustrates with the specific example of food allergies:

"*However, food is not unsafe merely because its inherent nutritional or chemical properties cause, or its inherent nature causes, adverse reactions only in persons with allergies or sensitivities that are not common to the majority of persons*."

Similar to the EU General Food Law, in the US there is the concept of "adulteration". Food is considered adulterated if it "*bears or contains any poisonous or deleterious substance which may render it injurious to health*" (FD&C Act,). This is commonly used as the basis for enforcement action in the US, and it is the legal responsibility of the relevant US agency, which for most foods is the Food and Drug Administration, to determine what constitutes "safe" (or injurious to health). This is usually undertaken by the agency as a case-by-case risk assessment which can be used in legal proceedings if the food business operator challenges the determination of "adulteration". In addition to this post-market control system there are a wide range of food specifications codified in US law, including standards of identity, permitted uses of various ingredients, and standards for processing etc. Beyond general laws in the US, the concept of "safe" and "safety" is defined in the context of food additives as the "*reasonable certainty in the minds of competent scientists that the substance is not harmful under the conditions of its intended use*". Other key legislative instruments in the US include the Food Safety Modernisation Act (FSMA, 2011), which introduced a risk-based approach to food manufacturing similar to the EU's (European Parliament, 2015). Within Food Safety Modernisation Act is the concept of "SAHCODHA hazards". These are hazards that are identified as presenting a "*reasonable probability that exposure to the hazard will result in serious adverse health consequences or death to humans or animals*" and are analogous to hazards which when present in marketed food would require a Class I recall because of a situation in which there is a reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death (https://www.fda.gov/medical-devices/postmarket-requirements-devices/recalls-corrections-and-removals-devices#2). This designation is used to determine the level of appropriate preventive controls in supply chains. In contrast, the United States Department of Agriculture refers to food safety in general as "*the conditions and practices that preserve the quality of food to prevent contamination and food-borne illnesses*" (https://ask.usda.gov/s/article/What-does-food-safety-mean).

In the EU, safety criteria for the assessment of foods meeting the legal definition of NF have been developed according to internationally established scientific principles and guidelines formulated through the work of the Organisation for Economic Co-operation and Development (OECD), Food and Agriculture Organisation (FAO), World Health Organisation (WHO) and the Codex Alimentarius Commission.

Key elements determining toxicological testing requirements include a review of the available data on the material and closely related materials, the history of consumption and knowledge of the source from which the food is derived, such as whether it is a potential source of toxins, anti-nutrients or known allergens, and a chemical analysis of its components. Depending on these determinations, conventional studies of toxicity, including (sub-chronic and) chronic toxicity, reproductive and developmental toxicity, genotoxicity and/or carcinogenicity, and other studies to examine specific biological processes that may not have been fully examined in the general studies, may need to be performed on the food components as appropriate. For most of these assessments, standards, methods and protocols have been clearly established. Although comprehensive guidance on allergenicity assessment for NF is provided, clear data requirements concerning this hazard are lacking, especially what evidence to provide on *de novo* allergenicity, leaving uncertainty with the applicants about what data to provide and how diverse types of data will be interpreted. The origins of the guidance in the GM foods model are still evident, and it is therefore not completely adapted to modified proteins or new protein sources, that will generally contain a complex mixture of many different proteins of which many will be consumed in higher amounts than new or modified proteins expressed in GM organisms.

Verhoeckx et al. (2016) proposed a strategy specific for the allergenicity assessment of the EU concept of NF proteins and protein sources. This incorporates history of intake, taxonomy, homology with other proteins, information on usage, and subsequently evaluating experimentally the capacity of the protein(s) to sensitise and elicit reactions. However, Verhoeckx et al. (2016) emphasise that environmental, geographic, demographic and cultural factors should also be considered in allergenicity assessment since a protein source can be considered as safe in some parts of the world while it is considered as unsafe on the grounds of allergenicity for at-risk populations in other parts. The strategy also briefly considers how the results of such an evaluation might be used to derive a safety prognosis through comparison with proteins of known allergenicity.

The above brief analysis of the concept of food safety and its implementation provides a key conclusion that it is multidimensional and highly contextual: different judgements could be reached for the same food or ingredient, depending on the risk management objectives driving the risk assessment.

4. Improved Allergenicity Risk Assessment Strategy – conclusions from the ImpARAS COST action

The COST Action ImpARAS, initiated and chaired by the Netherlands Organisation for Applied Scientific Research (https://www.cost.eu/actions/FA1402/), constituted one of the largest activities in the area of allergenicity assessment of novel and modified food proteins in recent years (Verhoeckx et al., 2020). Over 300 experts and representatives from industry, academia, risk assessment, risk management, regulatory and clinical bodies of 30 countries collaborated for 4 years and exchanged information and views on current strategies and methodologies, as well as limitations and improvements needed in the assessment of allergenicity of novel and modified food proteins. Operating through 4 working groups, ImpARAS addressed the current status of and future needs in the fields of protein chemistry and structure, *in vitro* and *in vivo* methods to predict sensitisation and allergy, and finally risk analysis. Outputs, achievements and recommendations of the Action are summarised in Verhoeckx et al. (2020).

A key insight of ImpARAS was the consensus that strategies and methods to assess the risk of allergic responses to novel and modified food proteins resulting from cross-reactivity with existing known allergenic proteins perform well. In contrast, strategies and methods to assess the potential of novel and modified food proteins to induce new food

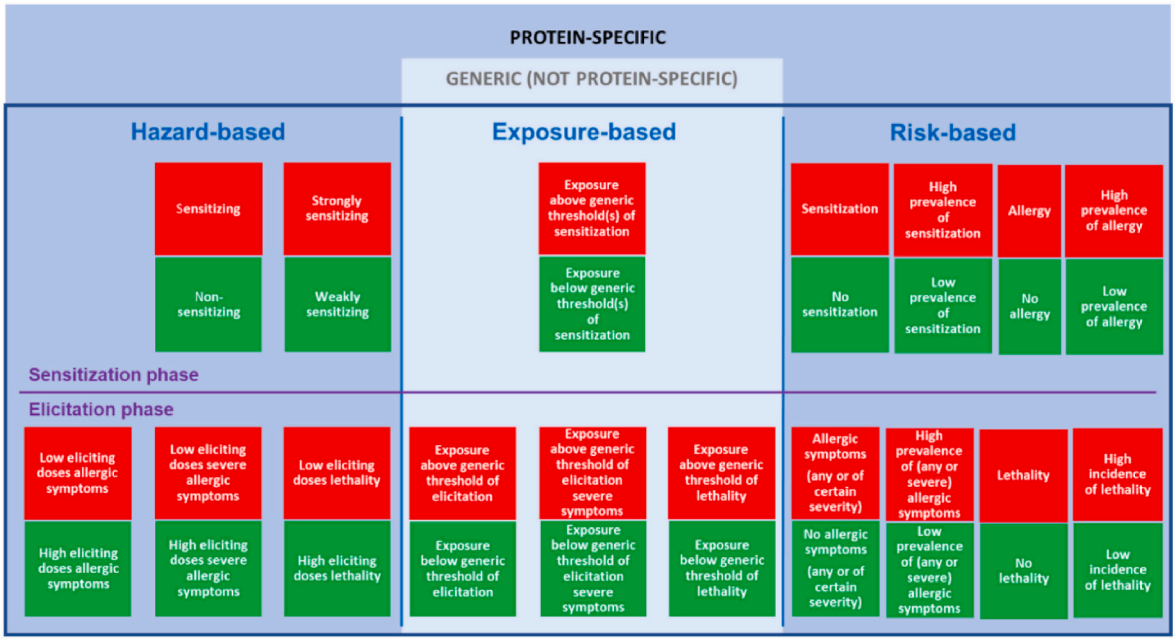

Fig. 2. Overview of potential parameters for allergenicity assessment for both the sensitisation and elicitation phases considering hazard-, exposure- and risk-based management objectives (reproduced with permission from Houben et al., 2019).

allergies, i.e., *de novo* sensitisation and allergenicity, are largely lacking. A dearth of guidance from the risk management sector regarding the parameters that should be assessed and the decision-making criteria which apply, forms a major hurdle for effective and efficient development of such strategies and methods. In order to help overcome these obstacles, the ImpARAS participants (Houben et al., 2019) argue that embarking upon an allergenicity risk assessment requires a strategy that is guided by the risk management questions and risk assessment goals defined by risk managers, an approach aligned with that of the Codex Alimentarius (CommissionCodex Alimentarius, 2003; FAO/WHO, 2007a; FAO/WHO, 2007b). Developing such a strategy, and the methods required to deploy the strategy, needs to be based on consensus and harmonisation over the criteria for risk management decision-making and parameters that the methods are called upon to assess. Without clarity on the risk management questions and risk assessment goals, method development will remain untargeted and a poor use of resources, entailing a higher cost of entry into the sector and inhibition of innovation.

An earlier International Life Sciences Institute (ILSI) Europe Expert Group considered possible parameters that might be used to enable the comparison between known allergenic foods as a basis for risk-based decision-making regarding prioritisation for regulation of existing allergenic foods based on public health relevance. They proposed to express allergenicity using a 2-dimensional matrix, with the prevalence of allergy to the allergenic foods forming one dimension and the potency (inverse of the eliciting dose) of these foods for triggering allergic symptoms in allergic subjects forming the other dimension. They presented a proof of principle for the expression and comparison of allergenicity between different foods based on these parameters.

In a paper published under the aegis of the COST Action ImpARAS, Houben et al. (2019) considered the applicability of these same parameters (i.e., prevalence of allergy within a population and potency for triggering symptoms) to the allergenicity assessment of novel and modified food proteins. The authors presented a proof of principle for the proposed approach based on existing research on the allergenicity resulting from cross-reactivity between shrimp proteins, a well-known allergenic protein source, and insect proteins, a potential food protein source new to many markets. Allergenicity data on insect proteins needed for this were generated using the research approach to assess allergenicity of complex food protein products as described by Verhoeckx et al. (2016) and Houben et al. (2016). Application of a similar approach for new potential allergens and *de novo* sensitisation and allergenicity would however pose difficulties as it requires data on both prevalence and potency, which are challenging to generate prospectively, prior to a food being consumed to a substantial extent. Houben et al. (2019) therefore acknowledged that parameters other than those useful for existing allergenic foods or for cross-reactivity might be more suitable in the context of the assessment of *de novo* sensitisation and allergenicity of novel and modified food. Therefore, they presented an exhaustive overview of other potential parameters for allergenicity assessment for both the sensitisation and elicitation phases considering hazard-, exposure- and risk-based management objectives (see Fig. 2).

The authors then described for each potential parameter the corresponding acceptance criteria that would permit attainment of the objective to be ascertained, and following from those, the type of methodology that would enable generation of the appropriate data. For instance, a food for which the hazard-based parameter "eliciting dose" was "*high*" could be considered to pose an acceptable risk whereas a food for which the value was "*low*" would be deemed to pose an unacceptable risk. This parameter implies the capacity to measure or predict eliciting dose prospectively and differentiate between "high" and "low", and therefore would require appropriate methodologies for this purpose. The extent and type of data needed to apply each of the parameters and criteria differ from option to option and each choice would also have its specific consequences regarding the risks that may or may not be tolerated. The paper also contains an inventory of implications for each optional parameter and criterion for risk management, data requirements and methods needed. With the work, the authors aimed to "*promote discussions between different stakeholders on how allergenicity could be better defined for the purpose of safety assessment*", without expressing their own preference for any of the options. Yet, the listed implications of each of the parameters for assessment and criteria for risk management clearly also have implications in terms of acceptability of the risk management consequences to stakeholders. Further, the implications regarding data requirements and methods needed for generating such data imply the development of suitable additional methods for some of the options. This clearly has implications for feasibility, time frame and resources needed for such development. In the following

section, we address the implications of risk management choices with respect to the parameters and criteria listed by Houben et al. (2019) from the perspective of societal acceptability and technological feasibility.

5. Societal acceptability and technological feasibility of inputs needed for risk management decision-making

Societal choices largely determine the societal acceptability of different possible risk management objectives and outcomes, hinging on the concept of tolerable risk. An ILSI-Europe Expert Group recently reviewed the concept and its application in the context of reference doses for regulated allergens (Madsen et al., 2020). The authors proposed a framework to facilitate the generation of a consensus around such decisions, a key element of which was the involvement of all relevant stakeholders. Such a framework could readily be adapted to help reach decisions on societal acceptability of different risk management outcomes for assessment of allergenicity of new or modified protein-containing food sources and ingredients. However, its application in this instance was beyond the scope and resources available to the group. Instead, a sub-group of authors of the present paper, representing different stakeholder groups (academia, consultancy and industry), reviewed each of the risk management outcomes relating to allergenicity listed by Houben et al. (2019). They evaluated their likely societal acceptability, technological feasibility and timescale needed for method development and judged feasibility based on likelihood that the technical requirements could be made operational within a 5-year timescale. These outcomes represent, of course, a specific subset of the factors influencing societal acceptability and exclude, for instance, considerations of sustainability, which are likely to gather increasing importance in the context of climate change. The conclusions of this subgroup were then presented to the whole authors group for critical review.

The acceptability of parameters and criteria partly depends on whether methods exist to assess the parameters of interest according to the chosen criteria now, or at least in the near future. These dependencies are reflected in historical EFSA Opinions regarding potential allergenicity of new food proteins, new protein sources or new applications of proteins in food. Three examples of EFSA Opinions were assessed by Houben et al. (2019) to illustrate the parameters and criteria that had been used: Chia (*Salvia hispanica* L.) seed and ground whole chia seed for use in bread, Rapeseed (*Brassica napus*) protein isolate for use in general foods, and ISP for use in ice creams (Houben et al., 2019). In all three cases, the risk management decision accepted a possible allergenic hazard. The decision was made based on an expectation of negligible risk, which in some cases was achieved through a requirement for product labelling e.g. rapeseed protein may cause allergic reactions in consumers who are allergic to mustard (2014/424/EU, 2014; Commission Implementing Decision, 2014/424/EU). Though, in all three cases a risk-based criterion was used, for ISP the criterion was applied to the sensitisation phase, with the conclusion that ISP is "*unlikely to sensitise potentially susceptible individuals*", implying that the risk of *de novo* sensitisation is considered negligible. Additionally, ISP was also considered unlikely to trigger allergic responses in individuals allergic to fish, i.e. a risk-based criterion applied to the elicitation phase. In the other two cases, the risk-based criterion applied to the elicitation phase was also used, which was mainly based on the absence of evidence in the literature for any allergic or cross-allergic responses after consumption (chia) or mitigating cross-reactive allergic responses in mustard-allergic individuals through labelling, and therefore avoidance by potentially vulnerable consumers (rapeseed). Remarkably, in these latter two cases, *de novo* sensitisation was considered possible according to the EFSA Opinion, but the ensuing risk was not clearly described or taken into consideration in the decision. The explicit acceptance of a possible allergenic hazard (and an implicit acceptance of the possible resulting risk) seemed associated with the acknowledgement that methods for assessing and excluding *de novo* sensitisation and allergenicity are currently lacking. The recent EFSA Opinion on mealworm (EFSA Panel on Nutrition, Novel Foods, Food, Allergens et al., 2021a) tends to reinforce this conclusion. It analyses in some detail the risk of *de novo* sensitisation by the product, as well as cross-reactivity with crustaceans and house dust mites, as detailed in (Broekman et al., 2016, 2017). While stating that people allergic to crustaceans should avoid consuming the product, the Nutrition, Novel Foods and Food Allergens (NDA) Panel concluded "*that the NF is safe under the proposed uses and use levels. In addition, the Panel notes that allergic reactions are likely to occur*." The recent EFSA Opinion on mung bean protein isolate (EFSA Panel on Nutrition, Novel Foods, Food, Allergens et al., 2021b), is illustrative of a similar acceptance of risk: "*Considering the information provided, this NF has the potential capacity to sensitise individuals and to induce allergic reactions (co-sensitisation or cross-reactivity) in individuals allergic to soybean, peanut, lupin as well as to birch pollen* ". The Panel concludes that the NF, mung bean protein, is "*safe under the proposed conditions of use*", without risk management measures being proposed for the allergic population at risk. It should be noted that the applicant did not perform any laboratory or animal test to assess the allergenicity of the NF. Instead, the data presented showed that amino acid sequence homologies between mung bean proteins and those of soybean, peanut and lupin were higher than 50% using the BLAST program in different databases.

To summarise, the above aspects all illustrate the importance of clarity on what risk we want to prevent, mitigate or are willing to accept, but also the fact that methodological feasibility plays a role in the ultimate choice of parameters to assess and the formulation of criteria for decision making. The analysis also illustrates the lack of guidance over what applicants are expected to provide to demonstrate safety from an allergenicity point of view, leaving what data to provide to their judgement.

Houben et al. (2019) catalogue an exhaustive inventory of possible hazards, exposures or risks that society might want to avoid or is willing to accept. These range from avoiding the possibility that people might die from or suffer severe allergic reactions to NF proteins or protein sources, to avoiding any possibility of sensitisation to such foods (and thereby avoid any adverse outcome of an allergic nature). They further considered the implications of each of the risk management objectives in terms of the constraints that they would impose on the introduction of novel proteins or protein sources as well as the methodological developments that they would require.

Improvements in the methodological repertoire will only be achieved and implemented if accepted by stakeholders, and scientifically and technologically feasible within an acceptable timeframe and resource deployment. As described in the first paragraph of this section, we reviewed the inventory of parameters and criteria and their implications described in Houben et al. (2019) against the criteria of societal acceptability and technological feasibility when applied to the assessment of allergenicity of novel and modified food proteins (Houben et al., 2019). Our underlying assumptions were that society would tolerate a low rate of sensitisation or allergy, if this allowed for innovation and new products, together with a 5-year delivery timeframe for method development. Results of this analysis are set out in Tables 2A and 2B. We limited ourselves to considerations applying to the pre-market assessment of *de novo* sensitisation and subsequent possible elicitation by novel and modified food proteins, because strategies and methods to assess the risk of allergenicity of novel and modified food proteins resulting from cross-reactivity with existing allergenic proteins are largely available and perform well, as already discussed.

Our analysis shows that out of 11 (7 for sensitisation, 4 for elicitation with possible distinctions between different effects) combinations of theoretically possible parameters and criteria for the assessment of allergenicity of novel and modified food proteins most (9) are expected to be unachievable, either because of low societal acceptability, or low probability of technological feasibility within an acceptable timeframe (5 years) and level of resources required, or both. We will discuss below

Table 2A

Sensitisation phase: parameters and criteria for risk management decision making before novel food enters food market (after Houben et al., 2019). For criteria used for societal acceptability and technological feasibility: see text section 5.

Criteria	Parameter(s)						
	Hazard-based		Exposure-based	Risk-based (derived from integration of hazard and exposure outcomes)			
Accepted	Non-sensitising	Weakly sensitising	Exposure below generic threshold of sensitisation*	No sensitisation expected	Low prevalence of sensitisation expected	No allergy expected	Low prevalence of allergy expected
Rejected	Sensitising	Strongly sensitising	Exposure above generic threshold of sensitisation*	Sensitisation expected	High prevalence of sensitisation expected	Allergy expected	High prevalence of allergy expected
Societal acceptability	Acceptability for safety high	Acceptability for safety high	Acceptability for safety high	Acceptability for safety	Potentially acceptable for safety	Acceptability for safety high	Acceptability for safety intermediate
	Highly constraining for the innovation of novel food products and therefore also sustainability (non–sensitising foods do not exist.	Consensus on suitable parameters to quantify sensitisers needed.	Scope of applicability limited to proteins present at low concentrations.	Highly constraining for the innovation of novel food products and therefore also sustainability (exception proteins present below threshold of sensitisation *(see exposure base criterion).*	Consensus on limits of acceptability low/high prevalence needed.	Outcome needs to be defined as the criterion could theoretically be falsified by a particular case. Translates into a distinction between proteins considered allergenic in practice (safety issue) and proteins not being allergenic in practice.	Consensus on suitable parameters to quantify allergy needed.
	Use of animal models no longer accepted.	Consensus on threshold to distinguish weak from strong sensitisers needed.	Consensus on threshold for safe exposure levels needed.				Consensus on limits of acceptability low/high prevalence needed.
Scientific and technological feasibility	Feasibility > 10 years	Feasibility > 10 years	Feasibility in 5 years:	Feasibility > 10 years	Feasibility > 10 years	Feasibility in 5 years: likely	Feasibility > 15 years
	Validated methods to measure sensitisation to novel foods are not available.	Appropriate parameters to define weak/strong sensitizers and validated methods to measure these parameters are not available.	Sensitisation data, consumption data and protein abundance in existing foods to develop TAC need to be collected.	Needed: exposure data of novel foods, information on sensitisation (see hazard-based criteria) and other influential factors.	Reliable test battery to measure sensitisation and exposure to predict prevalence of sensitisation are not available yet and are very complex.	New/emerging approaches such as *in silico* prediction models can be improved and applied in reasonable timeframe.	Predicting the outcome of hazard, exposure and other influential factors for the sensitisation phase will be very complex. We do not have a proof of principle for an approach.
	A scientific and technological solution could be achieved, but acceptance and implementation will take additional time.	A scientific and technological solution could be achieved, but acceptance and implementation will take additional time.	Feasibility is high for proteins with an exposure level below a generic threshold of sensitisation.	Predicting the outcome of hazard, exposure and other influential factors for the sensitisation phase will be very complex. Proving the absence of something, thus also of sensitisation, is impossible.	Understanding and integration of results with the influence of exposure is needed.	Application to foods with very large numbers of different proteins may be more difficult, but combination with exposure-based cut-offs could help (see exposure base criterion).	Establishing the prevalence of allergy as a benchmark for the development of methods has proven difficult for whole foods, so not to mention for individual proteins.
	Likely, such method would more distinguish different sensitising potencies rather than providing a binary +/- result.	Method development will only be effective if parameter and criterion for decision making are defined.		Possibly feasible for proteins at low levels (below a generic threshold of sensitisation, see exposure base criterion).			
Conclusion	Societal acceptability low	Societal acceptability: likely high	Societal acceptability: likely high	Societal acceptability: presumably low	Societal acceptability: likely high	Societal acceptability: likely high	Societal acceptability: likely high
	Technological feasibility: no or not within reasonable timeframe.	Technological feasibility: no or not within reasonable timeframe.	Technological feasibility: high, for proteins present at low levels; low for proteins present at nutritionally relevant levels.	Technological feasibility: low	Technological feasibility: low	Technological feasibility: reasonable, particularly in combination with exposure-based cut-offs.	Technological feasibility: low

the different options in more detail.

For hazard-based criteria for the sensitisation phase, two criteria were identified: whether sensitising or not and whether weakly or strongly sensitising. Society would accept both non-sensitising and weakly sensitising proteins (green in Table 2A and Fig. 2) because little or no sensitisation will be unlikely to lead to serious allergic reactions. However, the current paradigm stipulates that non-sensitising proteins do not exist. "Non-sensitising" as an acceptance criterion would therefore not be compatible with the needs of society in terms of new sources of protein and would hamper the introduction of NFs to the market and will thus be a threat to food security. "Weakly sensitising" as an acceptance criterion would be more acceptable but requires agreement on the parameters differentiating weakly from strongly sensitising proteins such as amount needed to sensitise, levels of specific IgE induced, frequency of sensitisation etc. Once such parameters are defined, a consensus must be obtained on a threshold that distinguishes weak from strong sensitisers, reflecting societal views on tolerable risk. This might be expressed as a situation where a normal pattern of consumption would result in an incidence of sensitisation not exceeding x%, but it might be more complex and require knowledge of the dose-response relationship between exposure, characteristics of exposure, and response, which may not be monotonic. Unfortunately, we, as society, also have difficulty in deciding on cut-offs where safety is concerned.

The scientific and technological feasibility of proving a negative, i.e., the absence of something, will always be difficult. In the current state of the art, a negative test for sensitisation in human beings (e.g. no specific IgE present in blood) is not necessarily conclusive proof of an absence of sensitising potential. Outcomes depend amongst other factors on the selected population (e.g. country, age), exposure (route) to the protein, and the form and condition of the protein (extracts, processing) used. Large prospective studies in humans are therefore technically and ethically challenging to design and run when investigating NFs, even ignoring the resources required. An approach derived from the Human Repeated Insult Patch Test (HRIPT), which is used in the context of demonstrating safety regarding the potential of a substance not to provoke allergic contact dermatitis could perhaps be adapted. This would

Table 2B
Elicitation phase: parameters and criteria for risk management decision-making before novel food enters food market (after Houben et al., 2019). ** With possible distinctions, for instance between any effects, e.g. severe effects and lethality versus any subjective effects. For criteria used for societal acceptability and technological feasibility: see text section 5.

Criteria	Parameter(s)			
	Hazard-based	Exposure-based	Risk-based	
Accepted	High eliciting dose**	Exposure below generic threshold(s) of elicitation**	No allergic symptoms expected**	Low incidence of allergic symptoms expected**
Rejected	Low eliciting dose**	Exposure above generic threshold(s) of elicitation**	Allergic symptoms expected**	High incidence of allergic symptoms expected**
Societal acceptability	Acceptability: uncertain Criterion implies acceptance of development of some sensitisation and/or allergy. Definition of cut-off values between high and low eliciting dose and agreement on them as well as on the type of allergic symptoms to cover needed.	Acceptability: uncertain Issues similar to those for the hazard-based criterion. Being able to discriminate between a threshold for any symptoms and a threshold for severe symptoms could improve acceptability.	Acceptability: uncertain Issues around acceptance of development of sensitisation and allergy similar to those for the hazard-based criterion. Apart from that, this criterion could likely be acceptable.	Acceptability: uncertain Issues around acceptance of development of sensitisation and allergy similar to those for the hazard-based criterion. Low/high would need to be defined quantitatively.
Scientific and technological feasibility	Feasibility: > 15 years Requires prospective prediction of eliciting dose for many individual proteins, currently not possible.	Feasibility: 15 years: may be possible through new methodologies. Requires knowledge of threshold of elicitation for individual proteins. This faces similar issues as determining eliciting dose prospectively, although perhaps to a less complex degree.	Feasibility: not within 10 years If exposure is not below a threshold of elicitation (see exposure-based criterion), prediction of the combined outcome of hazard, exposure and co-factors seems not feasible.	Feasibility: not for coming decade(s) If exposure is not below a threshold of symptom elicitation (see exposure-based criterion), prediction of the combined outcome of hazard, exposure and co-factors seems not feasible.
Conclusion	Societal acceptability: presumably low Technological feasibility: no or not within reasonable timeframe	Societal acceptability: possibly Technological feasibility: no or not within reasonable timeframe	Societal acceptability: possibly Technological feasibility: very low	Societal acceptability: possibly Technological feasibility: very low

be based on the principle of testing the hypothesis that protein in a novel food would not result in sensitisation above a defined protein-specific threshold, if eaten under conditions of use reflecting the range of usage among the exposed population.

Currently only animal models can be used to provide some measure of the sensitising potency of proteins (yes/no, weak/strong), although even then their domain of applicability for this purpose may be quite limited and difficult to generalise. Furthermore, their predictive value in relation to allergenicity risk assessment remains uncertain. The current models, some of which use adjuvants to induce sensitisation, are not validated for the purpose of measuring sensitising potency. Moreover, the ethical basis of animal tests generally and particularly in the context of food safety evaluation is questioned in many societies. Even though animal models have been developed (technological feasibility), scientifically this criterion is more challenging to address. Devising protocols appropriate to the task requires consideration of many factors, including but by no means limited to what species and strain to use, how many animals must be included, what parameters to evaluate (e.g. presence of specific IgE or other "allergic" antibody isotypes), which and how many doses to use, and processing aspects of products (Bogh et al., 2016). We judged that a scientific and technological solution might be achieved within 10 years, but acceptance and implementation would take additional time - easily up to 10 years or more. Likely, such method would probably distinguish between different sensitising potencies rather than providing a binary positive/negative result.

The third scenario related to sensitisation is exposure-based, namely exposure below and above a generic threshold of sensitisation. This criterion might be an acceptable option for proteins present at low levels (e.g. some GM products). For this parameter a consensus must be obtained on an exposure threshold of concern for allergic sensitisation (Threshold of Allergological Concern - TAC) below which a protein is effectively unable to sensitise and could therefore be exempted from further assessment of allergenicity if its abundance is low and the expected exposure remains below the threshold set. To define such a threshold, data on consumption, incidence of sensitisation and abundance of the (allergenic) proteins in the foods and the amounts of that food that are consumed are needed. These data can easily be obtained within a reasonable time horizon (up to 5 years) for existing allergenic foods. This option was indeed discussed by Working Group 4 (risk analysis) of the ImpARAS network (Houben et al., 2019) and considered potentially feasible, although development of the concept only reached a very early stage. Clearly, the intentional use of most new or modified proteins as foods would be far above any such threshold as they would aim to provide a nutritional contribution to the diet. The TAC for sensitisation would have value in enabling safety evaluation to focus on quantitatively significant proteins, present within a given food source or ingredient. The use of a TAC for sensitisation concept could be combined with *in silico* models (e.g. PREAL, AllerCatPro, AllergenFP, Random Forest) which can predict for allergenicity based on protein characteristics such as biochemical and physicochemical properties, sequential features, linear epitope patterns and subcellular locations (Dimitrov et al., 2014; Maurer-Stroh et al., 2019; Wang et al., 2013; Westerhout et al., 2019). An example of such an approach is the machine learning Random Forest model published by Westerhout et al. (2019). This successfully tested and validated model appeared able to distinguish allergenic from non-allergenic proteins, with a demonstrated sensitivity in identifying allergenic proteins of 91%, a specificity of 87% and an overall accuracy of 88%. The model was further shown to correctly predict two previously unknown proteins from insects as allergenic (Westerhout et al., 2019). Validation of the model using additional case studies like the insect proteins example would be welcome to strengthen confidence in the approach and to improve it further. The Random

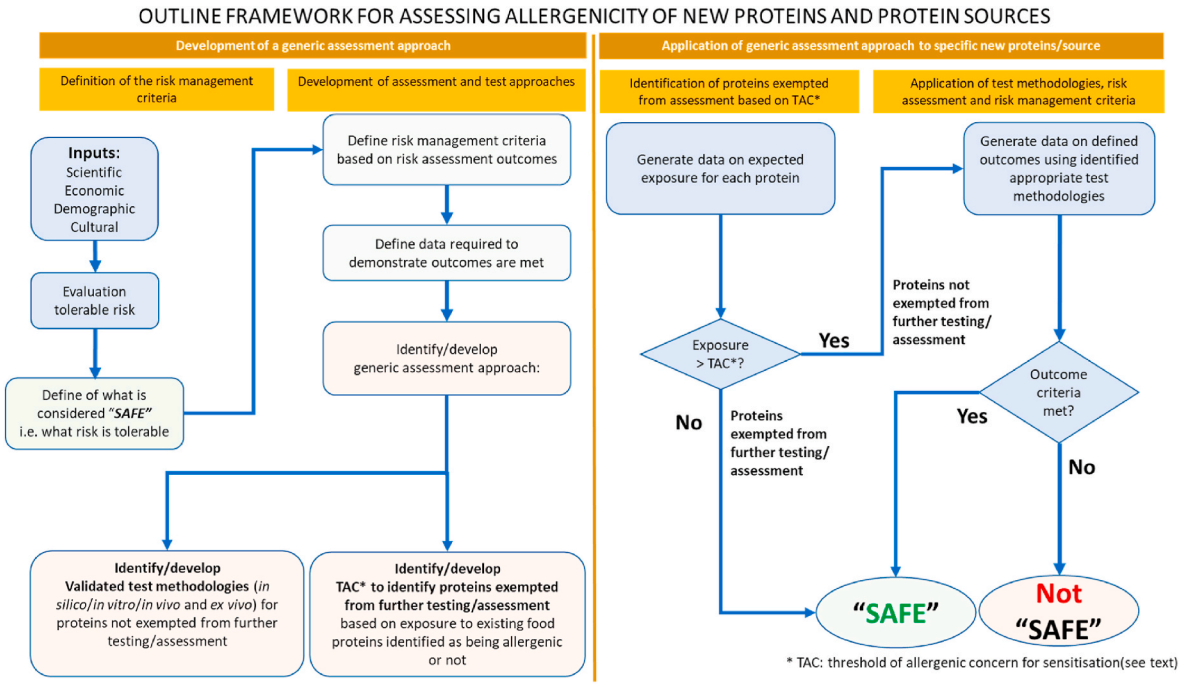

Fig. 3. Proposed framework for assessing allergenicity of new proteins and protein sources.

Forest model and other models have a limitation because they require amino acid sequence information for the proteins to be assessed, which may prove challenging for many processed and modified proteins or those that are complex mixtures from new sources containing large numbers of unknown or unidentified proteins. Combining this approach with the application of the TAC for sensitisation concept described above, would thus enable the assessment to focus on the more major proteins present, which are more easily identified using mass spectrometry. Furthermore, it must be noted that most *in silico* models include or totally rely on sequence alignment (completely or partly) with known allergenic proteins, which demonstrates sequence homology or similarity (e.g. AllerCatPro), a methodology which has been incorporated into allergenicity assessment guidelines as far back as the first iteration of the Codex Alimentarius guidance, published in 2003. This is not the case with the Random Forest model, PREAL and AllergenFP, which consider a multiplicity of properties pertaining to the protein(s) of interest to predict their allergenicity using a machine learning approach. Another significant difference between the models is the selection of allergenic and non-allergenic proteins for model building, which strongly influences the predicted outcomes (see also section 7). However, these *in silico* methods clearly have the potential to play an important role in the allergenicity prediction of novel proteins. This process will certainly be accelerated by the rapidly growing knowledge about artificial intelligence (AI), which makes it possible to combine and better understand even larger datasets obtained from *in silico, in vivo, in vitro* and clinical studies.

The risk-based scenarios in Table 2A are based on the predicted or expected risks of sensitisation and allergy development. These risks are determined by the sensitising capacity of the proteins (hazard) and the exposure to them. For all ***sensitisation***-related risk-based criteria it will likely be very complex to predict the outcome of hazard, exposure and other factors (e.g. environmental, age) influencing the sensitisation phase on the allergic status or health outcome. Furthermore, a reliable test (battery) for the hazard (sensitisation) is not available yet, as already discussed and many efforts have not resulted in a reliable test (see hazard-based criteria). Feasibility of developing a test (battery) within 10 years is judged low. Understanding and integration of results from such a test battery with the influence of exposure is needed for effective risk prediction but feasibility of the approach seems low for the coming decade(s) for most scenarios. For one scenario, namely "no allergy expected/allergy expected" we judged that societal acceptability would likely be high. Unlike sensitisation-based criteria, this criterion translates into a distinction between proteins being considered allergenic in practice (safety issue) and proteins not being allergenic in practice and could thus be applied to many proteins/protein sources where a history of exposure could be identified, with possible read-across to other related proteins, based on structural and functional attributes. However, for proteins with no history of exposure and or no basis for read-across, the inability to predict the occurrence and vigour of any allergic response would be a major obstacle, but machine-learning approaches such as Random Forest could provide a solution. This option thus seems at least partly feasible, e.g. based on a combination of methods, including machine learning approaches and application of a TAC for sensitisation.

Table 2B presents the possible parameters and criteria for risk management decision-making related to the ***elicitation*** phase. As in the sensitisation phase, in the elicitation phase we distinguish between hazard, exposure and risk-based criteria. For the elicitation phase we focus on the allergic symptoms. All criteria could be applied to mild, severe, or even lethal symptoms.

The societal acceptability of the hazard-based criteria high/low eliciting dose is uncertain because this criterion would imply acceptance of development of (some prevalence of) sensitisation and/or allergy. It is questionable whether this would (explicitly) be accepted. The idea of using eliciting doses as criterion can be envisaged. But this would likely need to be combined with exposure input, thus actually resulting in a risk-based criterion. The eliciting dose on its own is unlikely to be accepted as a criterion. Furthermore, severity or nature of symptoms would need to be defined if used as an element in this criterion.

Scientific and technological feasibility are low. It is unlikely that methods will be developed within a reasonable timeframe (<10 years) to predict the eliciting dose (ED) of individual proteins associated with different food allergy symptoms. Further, it would need knowledge of eliciting doses of large numbers of individual proteins as a benchmark to develop. It is unlikely that this information would become available within a foreseeable timeframe, based on the known difficulty of

developing such knowledge for whole foods.

In exposure and risk-based scenarios, the criteria could likely be acceptable. However, severe symptoms as well as low/high incidence would need to be defined if used in a criterion. For a criterion based on severe symptoms or even lethality, elicitation of mild symptoms or a certain incidence of severe symptoms or even lethality would need to be accepted, and it is questionable whether this would be acceptable.

For all elicitation-related risk-based criteria it will be very complex to predict the combined outcome of hazard, exposure and co-factors if exposure is not below a threshold of symptom elicitation (see exposure-based criterion). Scientific and technological feasibility is not foreseen for coming decades.

In conclusion, we did not find any scenarios which met our assumptions for being societally acceptable and technologically feasible for the elicitation phase. For the sensitisation phase, development, and application of a concept of TAC for sensitisation and bioinformatics approaches trained for distinguishing between proteins being considered allergenic in practice and proteins not being allergenic in practice, and particularly a combination of these, might provide an acceptable and feasible approach.

6. Developing a framework for evaluating allergenicity resulting from cross-reactivity of, and *de novo* sensitisation by, new or modified protein-derived foods

Any strategy aiming to assess the allergenicity of new or modified protein-containing food sources and ingredients ultimately seeks to establish whether such foods are safe regarding that particular hazard. As discussed in section 3, safety is not an absolute property of a food, but rather references concepts such as tolerability/acceptability of any risk, how the food is used as well as any information provided to consumers, such as via labelling. The relative nature of safety and the need for stakeholders to be involved in formulating what is considered as safe by society highlights how critical it is that the risk management question informs the development of the test strategy as discussed in the preceding section and elaborated in more detail in Houben et al. (2019). Fig. 3 outlines the main elements of an assessment framework formally integrating the risk management question(s) as the driver of the testing strategy i.e., as the hypothesis-generating mechanism underlying the testing programme. Assessing the potential allergenicity of a complex food containing many different proteins is different from assessing a single protein (e.g. ISP) or a small number of proteins. The proposed framework therefore comprises a key element to simplify the task, namely the application of a TAC for sensitisation [to be developed] to limit the number of proteins requiring an individual assessment.

The Framework consists of two sections: one describes the elements of the development of a generic risk assessment approach, while the other describes how the approach would be applied. Application includes the use of a TAC (for sensitisation) and drives the need for consideration of exposure in the development of the approach. Also, potential sensitisation through routes other than the gastrointestinal tract should be considered (e.g. work related respiratory or skin exposure). As mentioned before, reliable test methods are not available to assess the hazard of *de novo* sensitisation. However, the following suggestions for a testing strategy can be made based on the current level of knowledge (Box 1).

The text below describes the current gaps in these approaches and the further needs to develop more mature and validated testing approaches.

7. Gaps

Development and application of the proposed framework requires that a number of gaps shall be addressed. These fall into two categories, one concerns the range of acceptable risk assessment outcomes, while the other concerns technical and methodological issues, although there is overlap in any gaps in many respects.

7.1. Considerations of risk acceptability

In reviewing the risk management outcome criteria for both sensitisation and elicitation, we judged that acceptable outcomes were those resulting in limited public health impacts (expected low prevalence of sensitisation or allergy symptoms), yet still maintaining the possibility of product development and market entry. However, in the absence of numerical ranges, these do not necessarily provide the intended "level playing field" which developers of products require since they imply judgements regarding what constitutes, for instance, "low". The lack of such numerical values therefore constitutes a gap, although it may be deduced from some assessments (e.g. EFSA Opinions) that an expectation of a prevalence of reactions no greater than that associated with existing equivalent allergenic foods could be at least a starting point, although the lack of data to inform this approach constitutes a gap. However, it is presumably not acceptable to introduce a new product that is as allergenic as peanut. For such foods, values could be derived for risk management outcomes associated with cross-reactivity but would need to consider exposure as one confounding variable. An important drawback of such an approach is that it would be difficult and costly to implement prospectively as part of pre-market assessment. In the current state of knowledge, it would also raise ethical issues as it would involve a degree of human exposure. It should therefore come coupled with proposals for post-market monitoring that could demonstrate the validity of the pre-market assessment.

7.2. Technical and methodological issues

Other knowledge gaps are limited quantitative data on parameters (e.g. timing and route of exposure), which influence vigour of sensitisation. In fact, there is increasing evidence that sensitisation to food proteins can, or perhaps does mainly occur via other routes than the gastrointestinal tract after ingestion (Brough et al., 2020). It is now known that sensitisation may also occur via exposure through the skin and respiratory tract, and that exposure via the gastrointestinal tract may instead favour the induction of oral tolerance. Hence, there is a great gap in our knowledge on the delicate balance between sensitisation and tolerance induction, and just as the dose-response relationship

Box 1
potential approaches to assess *de novo* sensitisation

I. ***In silico or Artificial Intelligence (AI)***: bioinformatics approaches using protein characteristics: with single identified proteins or AI to combine and analyze complex datasets (*in silico*, *in vivo*, *in vitro*, clinical) to identify allergenic proteins.
II. ***In vitro***: models based on the MIE (Molecular Initiating Events) & KE (Key Event) concepts from the Adverse Outcome Pathway (AOP) for sensitisation (Lozano-Ojalvo et al., 2019; van Bilsen et al., 2017) such as uptake over mucosal barrier or epithelium activation with complete testing material or selected proteins.
III. ***In vivo/ex vivo*****:** studies with humans and/or animal studies

for sensitisation is poorly defined, there is a knowledge gap on a dose-response relationship for tolerance. Perhaps a deeper scrutiny of current immunotherapeutic approaches would yield dividends.

Technical and methodological gaps include identification of the TAC for sensitisation, which will require defining the appropriate metrics and knowledge sources to answer the question regarding levels of exposure that do not result in sensitisation, and possibly subdividing allergenic proteins into different categories. Understanding the sensitisation dose-response relationship, the role of other routes of exposure, besides the oral route in sensitisation (i.e., respiratory and dermal) and defining the metrics to measure it would contribute to filling this gap, as would further development and population of AOP and assays supporting those models. Animal models could prove of value in addressing some of the gaps. A lack of biomarkers of allergy outcomes which could serve to build a comparative scale of sensitisation potency also constitutes a gap, which might be addressed through revised or new AOPs.

Another gap concerns the allergen databases used to compare sequences (cross-reactivity) and to build *in silico* prediction tools for *de novo* sensitisation. The allergen sequence databases currently in use (e.g. WHO/IUIS Allergen Nomenclature, AllergenOnline.org, COMPARE, Allergome, AlgPred2.0) for the allergenicity risk assessment do not provide systematic information on the allergenic potential (minor/major allergen) or clinical relevance of entries and the inclusion criteria used are often different between databases. Moreover, not all databases are regularly updated (Mazzucchelli et al., 2018; Radauer and Breiteneder, 2019). This leads to large discrepancies in the quantity and quality of entries between existing databases (Maurer-Stroh et al., 2019). In addition, these databases do not distinguish between primary sensitising and cross-reactive allergens and contain fragments and isomers the clinical relevance of which is not always clear. Additional research is needed (e.g. inhibition studies) to uncover this information. The aforementioned aspects may hamper the development of reliable and predictive *in silico* tools for allergenicity prediction.

8. Priorities

For the development and application of the proposed framework we need to:

- Get consensus on what is considered a tolerable risk for allergenicity, sensitisation and elicitation, based on currently available methods for evaluating those outcomes. Principles discussed by Madsen et al. (2020) could provide a basis for this (see also section 5).
- Update allergen databases by harmonising inclusion criteria and only including clinically relevant allergenic proteins. Distinguish between primary sensitisers and cross-reactive proteins, minor/major allergens, and what to do with fragments and isomers, and include epitope information.
- Development of the TAC for sensitisation.
- AOP optimisation to address route of sensitisation, and tolerance induction, including the possible effect of environmental factors.
- While the scientific approach is further developed and optimised to assess the allergenicity of new or modified food protein sources, resources should be invested to develop robust and harmonised post launch/post market monitoring of new foods, to capture a potential new allergen or an increase in existing allergen prevalence.

9. Conclusion

The allergenicity assessment of new or modified proteins, is currently partly possible, covering only the part where similarity is assessed with known allergens, assessing the potential for cross-reactivity. This new identified allergen can then be managed in an equivalent manner to existing known allergens, factoring in wider considerations, such as public health impact. However, the assessment of *de novo* sensitisation and subsequent allergy is hampered by a lack of predictive and validated tools, as well as a lack of consensus by risk managers of what is considered a tolerable risk in the area of allergenicity. In this paper, several scenarios have been discussed where technical feasibility should be within reach and be acceptable from a societal point of view. A potential framework is proposed where the application of a TAC for sensitisation could be used as a tool to prioritise proteins of which exposure would exceed this threshold and that need further assessment. The next steps include are 1) obtaining stakeholder consensus on tolerable risks for allergenicity, (2) updating of allergen databases, (3) optimising the AOP of food allergy and based on this, development of appropriate methods and approaches to predict the likelihood and vigour of *de novo* sensitisation. While anticipating these scientific developments, investments in harmonised and robust post launch/post market monitoring can serve as an important source of information to accompany the launch of new or modified proteins, in the race for more sustainable sources to support global food security.

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CRediT authorship contribution statement

R.W.R. Crevel: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **K. Verhoeckx:** Writing – review & editing, Writing – original draft. **K.L. Bøgh:** Writing – review & editing, Investigation. **N. Buck:** Writing – review & editing. **A. Chentouf:** Writing – review & editing. **S. Flanagan:** Writing – review & editing. **M. Galano:** Writing – review & editing, Writing – original draft. **J.A. Garthoff:** Writing – review & editing. **S. Hazebrouck:** Writing – review & editing. **R. Yarham:** Writing – review & editing. **G. Borja:** Project administration. **G. Houben:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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Data availability

No data was used for the research described in the article.

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References

Bogh, K.L., van Bilsen, J., Glogowski, R., Lopez-Exposito, I., Bouchaud, G., Blanchard, C., O'Mahony, L., 2016. Current challenges facing the assessment of the allergenic capacity of food allergens in animal models. Clin. Transl. Allergy 6, 21. https://doi.org/10.1186/s13601-016-0110-2.

Broekman, H., Verhoeckx, K.C., den Hartog Jager, C.F., Kruizinga, A.G., Pronk-Kleinjan, M., Remington, B.C., Knulst, A.C., 2016. Majority of shrimp-allergic patients are allergic to mealworm. J. Allergy Clin. Immunol. 137 (4), 1261–1263. https://doi.org/10.1016/j.jaci.2016.01.005.

Broekman, H., Knulst, A.C., den Hartog Jager, C.F., van Bilsen, J.H.M., Raymakers, F.M. L., Kruizinga, A.G., Verhoeckx, K.C.M., 2017. Primary respiratory and food allergy to mealworm. J. Allergy Clin. Immunol. 140 (2), 600–603 e607. https://doi.org/10.1016/j.jaci.2017.01.035.

Brough, Helen A., Nadeau, Kari C., Sindher, Sayantani B., Alkotob, Shifaa S., Chan, Susan, Bahnson, Henry T., Lack, Gideon, 2020. Epicutaneous sensitisation in the development of food allergy: what is the evidence and how can this be prevented? Allergy 75 (9), 2185–2205. https://doi.org/10.1111/all.14304.

1-1969, CAC/RCP. (Adopted in 1969. Amended in 1999. Revised in 1997, 2003, 2020. Editorial Corrections in 2011). General principlea of food hygiene CAC/RCP 1-1969 In..

OJ L 196 C.F.R Commission Implementing Decision of 1 July 2014 Authorising the Placing on the Market of Rapeseed Protein as a Novel Food Ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (Notified under Document C(2014) 4256), 2014. Available at: https://eur-lex.europa.eu/legal-content/NL/TXT/?uri=CELEX%3A32014D0424.

Commission, Codex Alimentarius, 2003. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy 30 June–5 July, 2003. Appendix III, Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants and Appendix IV, Annex on the Assessment of Possible Allergenicity, pp. 47–60.

Council, Institute of Medicine and National Research, 1998. Ensuring Safe Food: from Production to Consumption. The National Academies Press, Washington, DC.

Dimitrov, I., Naneva, L., Doytchinova, I., Bangov, I., 2014. AllergenFP: allergenicity prediction by descriptor fingerprints. Bioinformatics 30 (6), 846–851. https://doi.org/10.1093/bioinformatics/btt619.

Dominique, T., Bresson, J.L., van Loveren, H., EFSA Panel on Dietetic Products, Nutrition, Products, Allergies, Nutrition, Allergies, 2021. Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/22831 (Revision 1)2. EFSA J. 19 (3), e06555 https://doi.org/10.2903/j.efsa.2021.6555.

EFSA NDA Panel, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA J. 14 (11), 4594. https://doi.org/10.2903/j.efsa.2016.4594.

Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Knutsen, H.K., EFSA Panel on Nutrition, Novel Foods, Food, Allergens, 2023. Safety of whole seeds of oilseed rape (*Brassica napus* L emend. Metzg.) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 21 (1), e07706 https://doi.org/10.2903/j.efsa.2023.7706.

EFSA Panel on Nutrition, Novel Foods, Food, Allergens, Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K.I., Knutsen, H.K., 2021a. Safety of dried yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 19 (1), e06343 https://doi.org/10.2903/j.efsa.2021.6343.

Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Knutsen, H.K., EFSA Panel on Nutrition, Novel Foods, Food, Allergens, 2021b. Safety of mung bean protein as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 19 (10), e06846 https://doi.org/10.2903/j.efsa.2021.6846.

European Parliament, 2015. Ecologic Institute and BIO intelligence Service. ENVI Food Safety Policy and Regulation in the United States, IP/A/ENVI/2015-05, PE 536.324 available at: http://www.europarl.europa.eu/RegData/etudes/STUD/2015/536324/IPOL_STU(2015)536324_EN.pdf.

FAO/WHO, 2007a. Working Principles for Risk Analysis for Food Safety for Application by Governments (Codex Alimentarius), first ed. Retrieved from.

FAO/WHO, 2007b. Working Principles for Risk Analysis for Food Safety for Application by Governments (Codex Alimentarius) - First Edition. Available at: https://www.fao.org/documents/card/en/c/fdaaa09d-8a3f-50c6-b801-945ffcac73a2/.

FD&C Act, Chapter 9 - Federal Food, Drug, and Cosmetic Act (Sections 301 - 399d), Sec. 402; Sec. 342- Adulterated Food.

Food Standard Code. Food standards Australia New Zealand. https://www.foodstandards.gov.au/code/Pages/default.aspx. (Accessed 17 February 2022).

Full Text of the Food Safety Modernization Act (FSMA), 111th Congress Public Law 353 C.F.R., 2011.

Houben, G., Burney, P., Chan, C.H., Crevel, R., Dubois, A., Faludi, R., Ronsmans, S., 2016. Prioritisation of allergenic foods with respect to public health relevance: report from an ILSI Europe food allergy task force expert group. Food Chem. Toxicol. 89, 8–18. https://doi.org/10.1016/j.fct.2015.12.028.

Houben, G., Blom, M., Alvito, P., Assuncao, R., Crevel, R., Faeste, C.K., Constable, A., 2019. Defining the targets for the assessment of IgE-mediated allergenicity of new or modified food proteins. Food Chem. Toxicol. 127, 61–69. https://doi.org/10.1016/j.fct.2019.02.036.

Lozano-Ojalvo, Daniel, Benedé, Sara, Antunes, Celia M., Bavaro, Simona L., Bouchaud, Grégory, Costa, Ana, van, Bilsen, Jolanda, H.M., 2019. Applying the adverse outcome pathway (AOP) for food sensitisation to support in vitro testing strategies. Trends Food Sci. Technol. 85, 307–319. https://doi.org/10.1016/j.tifs.2019.01.014.

Madsen, C.B., van den Dungen, M.W., Cochrane, S., Houben, G.F., Knibb, R.C., Knulst, A. C., Crevel, R.W.R., 2020. Can we define a level of protection for allergic consumers that everyone can accept? Regul. Toxicol. Pharmacol. 117, 104751 https://doi.org/10.1016/j.yrtph.2020.104751.

Maurer-Stroh, S., Krutz, N.L., Kern, P.S., Gunalan, V., Nguyen, M.N., Limviphuvadh, V., Gerberick, G.F., 2019. AllerCatPro-prediction of protein allergenicity potential from the protein sequence. Bioinformatics 35 (17), 3020–3027. https://doi.org/10.1093/bioinformatics/btz029.

Mazzucchelli, G., Holzhauser, T., Cirkovic Velickovic, T., Diaz-Perales, A., Molina, E., Roncada, P., Hoffmann-Sommergruber, K., 2018. Current (food) allergenic risk assessment: is it Fit for novel foods? Status Quo and identification of gaps. Mol. Nutr. Food Res. 62 (1) https://doi.org/10.1002/mnfr.201700278.

Mullins, E., Bresson, J.L., Dalmay, T., Dewhurst, I.C., Epstein, Michelle M., Moreno, F.J., EFSA Panel on Genetically Modified Organisms, 2022. Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. EFSA J. 20 (1), e07044 https://doi.org/10.2903/j.efsa.2022.7044.

Radauer, Christian, Breiteneder, Heimo, 2019. Allergen databases—a critical evaluation. Allergy 74 (11), 2057–2060. https://doi.org/10.1111/all.13841.

Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on Novel Foods, Amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and Repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (Text with EEA Relevance), 2015. OJ L 327 C.F.R.

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 Laying Down the General Principles and Requirements of Food Law, Establishing the European Food Safety Authority and Laying Down Procedures in Matters of Food Safety, 2015. OJ L 31 C.F.R.

Remington, B., Broekman, H.C.H., Blom, W.M., Capt, A., Crevel, R.W.R., Dimitrov, I., Constable, A., 2018. Approaches to assess IgE mediated allergy risks (sensitisation and cross-reactivity) from new or modified dietary proteins. Food Chem. Toxicol. 112, 97–107. https://doi.org/10.1016/j.fct.2017.12.025.

van Bilsen, J.H.M., Sienkiewicz-Szlapka, E., Lozano-Ojalvo, D., Willemsen, L.E.M., Antunes, C.M., Molina, E., Roggen, E.L., 2017. Application of the adverse outcome pathway (AOP) concept to structure the available in vivo and in vitro mechanistic data for allergic sensitisation to food proteins. Clin. Transl. Allergy 7, 13. https://doi.org/10.1186/s13601-017-0152-0.

Verhoeckx, K., Broekman, H., Knulst, A., Houben, G., 2016. Allergenicity assessment strategy for novel food proteins and protein sources. Regul. Toxicol. Pharmacol. 79, 118–124. https://doi.org/10.1016/j.yrtph.2016.03.016.

Verhoeckx, K., Lindholm Bogh, K., Constable, A., Epstein, M.M., Hoffmann Sommergruber, K., Holzhauser, T., Crevel, R., 2020. COST Action 'ImpARAS': what have we learnt to improve food allergy risk assessment. A summary of a 4 year

networking consortium. Clin. Transl. Allergy 10, 13. https://doi.org/10.1186/s13601-020-00318-x.

Wang, Jing, Zhang, Dabing, Li, Jing, 2013. PREAL: prediction of allergenic protein by maximum Relevance Minimum Redundancy (mRMR) feature selection. BMC Syst. Biol. 7 (5), S9 https://doi.org/10.1186/1752-0509-7-S5-S9.

Westerhout, J., Krone, T., Snippe, A., Babe, L., McClain, S., Ladics, G.S., Verhoeckx, K.C., 2019. Allergenicity prediction of novel and modified proteins: not a mission impossible! Development of a Random Forest allergenicity prediction model. Regul. Toxicol. Pharmacol. 107, 104422 https://doi.org/10.1016/j.yrtph.2019.104422.