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Defining the targets for the assessment of IgE-mediated allergenicity of new or modified food proteins

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ABSTRACT

Many food innovations rely on the introduction and use of new or modified proteins. New or modified food proteins may lead to major health risks due to their inherent potential to cause food allergy. Currently, the pre-market allergenicity assessment for new or modified food proteins and protein sources relies on methods for identifying allergenic hazards based on characteristics of known allergens. However, there is no general consensus on the allergenicity parameters to use and the criteria that should apply for the evaluation and decisions to be made. In this paper, we propose that the strategy for allergenicity risk assessment of new or modified food proteins and the methodologies applied should be governed by the risk management questions to be answered, reflected in the information needed by risk managers to enable their informed decision making. We generated an inventory of health outcome-related assessment parameters and criteria potentially important for risk management decision-making and we discuss the implications of selecting different optional criteria (e.g. cut-off values) for what could be accepted as safe with regards to the health outcomes in the (at risk) population. The impact of these various options on both method development and risk management practices was investigated.

1. Introduction

Securing our food supply, managing the environmental impact of food production, adapting to changing climate conditions, improving our health, and reducing the risk of disease development, require innovations in food sources, production and products. Many of such innovations rely on the introduction and use of new or modified proteins. For over 20 years, new food sources and products may only be placed on the EU market when safe in production and use (Commission, 1997a, b; EFSA Panel on Dietetic Products, 2016). New or modified food proteins, however, could eventually lead to major health risks due to their inherent potential to cause IgE-mediated allergic reactions in food allergic consumers or sensitization in consumers susceptible to the development of IgE-mediated food allergy (mainly atopic subjects).

With a current prevalence of about 3%, IgE-mediated food allergy is one of the most prevalent disorders in the Western world (Nwaru et al., 2014a, 2014b). It is a major cause of loss of health and loss of quality of life for allergic persons and their relatives, a frequent cause of (emergency) hospital admission, and its economic impact is immense; for instance, between 30 and 50% of all food recalls in the EU and US are allergen-related (Blom et al., 2018; Bucchini et al., 2016; FDA, 2016; Michelsen-Huisman et al., 2018; Versluis et al., 2015). Therefore, it is essential that innovations in food should not add to society's existing food allergy burden.

The EU Cost Action ImpARAS has built an interdisciplinary network of more than 300 scientists from about 30 countries with the aim to discuss and propose approaches to improve the allergenicity risk assessment of new and modified food proteins and protein sources

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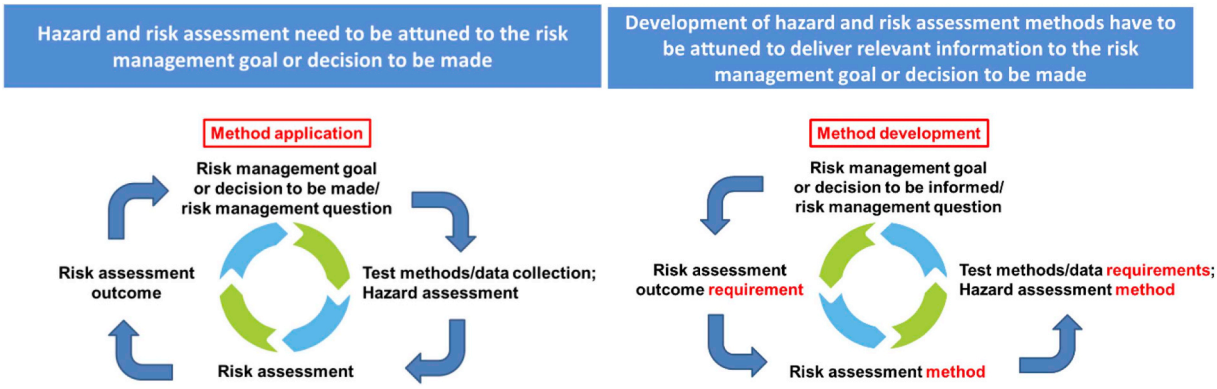

Fig. 1. Hazard and risk assessment methods should be appropriate to/aligned with risk management goals or questions.

(http://imparas.eu/). Currently, the pre-market allergenicity assessment for new or modified food proteins and protein sources relies on methods for identifying allergenic hazards based on characteristics of known allergens (Remington et al., 2018). However, there is no general consensus on the allergenicity parameters to use and the criteria that should apply for the evaluation and decisions to be made. In this paper, we propose that the strategy for allergenicity risk assessment of new or modified food proteins and the methodologies applied should be governed by the risk management questions to be answered, based on the decisions, which risk managers need to take. Thus, it is critical to first define which hazard or risk parameters must be assessed that, in turn, will enable the development and use of relevant methodologies. Furthermore, we discuss the implications of selecting different optional criteria (e.g. cut-off values) for what could be accepted as safe with regards to the health outcomes in the (at risk) population. Preferred methods, desired targets and decision criteria enabling potential allergenicity risk assessment and risk management of new or modified food proteins should be clearly defined, and preferably should be developed within the framework of an international collaboration. An ImpARAS working group has generated an inventory of health outcome-related assessment parameters and potential criteria important for risk management decision-making. The impact of these various options on both method development and risk management practices was investigated. This paper presents this work and aims to promote discussions between different stakeholders on how allergenicity could be better defined for the purpose of safety assessment, supporting and informing risk managers commissioned with the approval of new or modified protein-containing food products.

2. Risk analysis and the relationship between risk assessment and risk management

In Europe, Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 lays down the general principles and requirements of food law, the establishment the European Food Safety Authority, and procedures in matters of food safety (Commission, 2002). In this regulation, “risk analysis” is defined as the process consisting of three interconnected components: risk assessment, risk management and risk communication; “risk assessment” means a scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation; “risk management” means the process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options. The latter may include risk communication. Furthermore, Article 6 of the Regulation states that the “risk assessment shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner”. Slightly different definitions may apply in other frameworks, e.g. by WHO-IPCS (IPCS, 2004), but the terms and principles of risk analysis apply rather generally to all kinds of situations and domains and have already been applied to chemical and microbiological food safety issues for many decades. While an ILSI Europe Expert Group has defined distinct but interconnected risk analysis processes for each of the sensitization and elicitation phases of IgE-mediated food allergy (Houben et al., 2016), legislated food allergy risk analysis is rudimentary in nature, when compared to other fields.

As indicated above, risk assessment and risk management are not independent of each other but are interacting and iterative processes within the overall risk analysis. A risk assessment needs to be attuned to and to answer the risk management questions and needs. Therefore, the hazard and risk assessment methods applied and the information required from the risk assessment are determined by the risk management goal or decision to be made. Consequently, if new methods for hazard and risk assessment are to be developed, the methods, the information needed and the requested outcomes should be aligned with the information that is going to be requested for the risk management goal or decision to be made (Fig. 1.). The closer the fit between the information provided by the risk assessment and that required by the risk management question, the better and more effectively will the latter be addressed.

To define the requirements of new test and assessment methods, it is important to establish the test endpoints and in which way these contribute to the risk assessment. What is it that we need to know about new food proteins? This depends on the ultimate risk management decision that is to be made and the problem formulation that is given to risk assessors. What is the description of the risk that we want to manage or that we want to prevent? As illustrated later, at one extreme, we might want to prevent anyone becoming sensitized to a new protein, while at the opposite extreme we may accept that, rarely, people might experience fatal reactions. Fig. 2 illustrates the relationship between risk management decision-making, risk assessment outcome, and risk assessment process. It shows how the key parameter(s) and criterion (or criteria) for risk management decision-making, and thus the required outcome of the risk assessment (XXX in Fig. 2), play a central role in the risk analysis process.

The parameter(s) and decision criterion (or criteria) for risk management decision-making and thereby the information requested as an outcome of the risk assessment (XXX in Fig. 2) may be qualitative or quantitative. This paper does not intend to propose or give a preference for the parameter(s) and decision criterion (or criteria) XXX, but intends to present and discuss the different possible outcome scenarios with respect to IgE-mediated allergenicity of new or modified food proteins and their implications for risk management and for the development of methods and data needed for hazard and risk assessment.

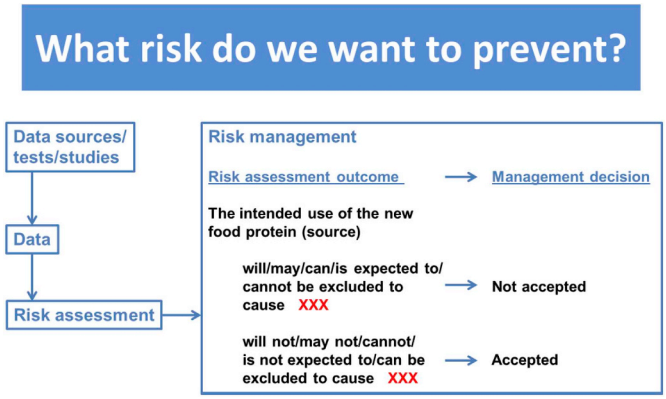

Fig. 2. The risks analysis process organized around the key parameter(s) and criterion (or criteria) for risk management decision-making.

3. Possible parameters and criteria for risk management decision-making

Based on the various mechanistic steps and progression in the development of IgE-mediated food allergy, an overview of possible (theoretical) parameters and criteria for risk management decision-making with respect to IgE-mediated allergenicity of new or modified food proteins has been composed (Fig. 3).

Possible risk management approaches include hazard-based, risk-based and exposure-based approaches. Thus, the scheme in Fig. 3 covers hazard parameters (based on the potential of the substance to induce harm, e.g. being a sensitizer or not, a strong sensitizer or not, having low eliciting dose or not), risk parameters (based on adverse events in subjects affected by certain levels of exposure to the hazard, e.g. prevalence of sensitization, incidence of allergic symptoms) as well as exposure-related parameters. Although often not a preferred choice, hazard-based approaches can be valuable in certain cases, for instance when the influence of exposure is not well understood (e.g. in the sensitization phase), when exposure does not have a clear influence, or in situations where stakeholders would like to prevent exposure to substances with specific properties (e.g. mutagenic, teratogenic or sensitizing chemicals). The options shown in Fig. 3 provide also for the potential application of an exposure-based approach based on a Threshold of Allergenic Concern (TAC) concept, comparable to the Threshold of Toxicological Concern (TTC) concept (Kroes et al., 2004; Leeman et al., 2014; Munro et al., 1996, 2008; Rennen et al., 2011). The feasibility of the development and application of a TAC concept for the sensitization and/or elicitation phase in IgE-mediated food allergy is currently being explored by an ImpARAS working group and are not discussed further here. Decisions based on the prevalence of sensitization or allergy or on the incidence of allergic symptoms could be based on low (accepted) versus high (not accepted) prevalence or incidence or on absence (accepted) versus presence (not accepted). Additional or other distinctions may be considered such as, for instance, a separate criterion of life-threatening reactions or differences in age groups. Risk management decision-making could be based on a single parameter/criterion or on combinations of parameters/criteria. A combination could be, for instance: a low prevalence of food allergy (below a defined level) and absence of life-threatening reactions. The presented options should not be understood as a decision tree, as a parameter does not necessarily depend on another parameter, even if it occurs later in the disease cascade. For instance, if risk managers or regulators decided that new or modified protein products which require very high doses in order to elicit allergic symptoms would pose an acceptable risk under all circumstances, it would not be necessary to assess the potential prevalence of sensitization or allergy to said proteins or foods.

4. Applying the risk analysis methodology

The different parameters and criteria presented in Fig. 3, or possible extensions of those as discussed above, can in principle be applied both to assessing potential cross-reactivity of new or modified food proteins

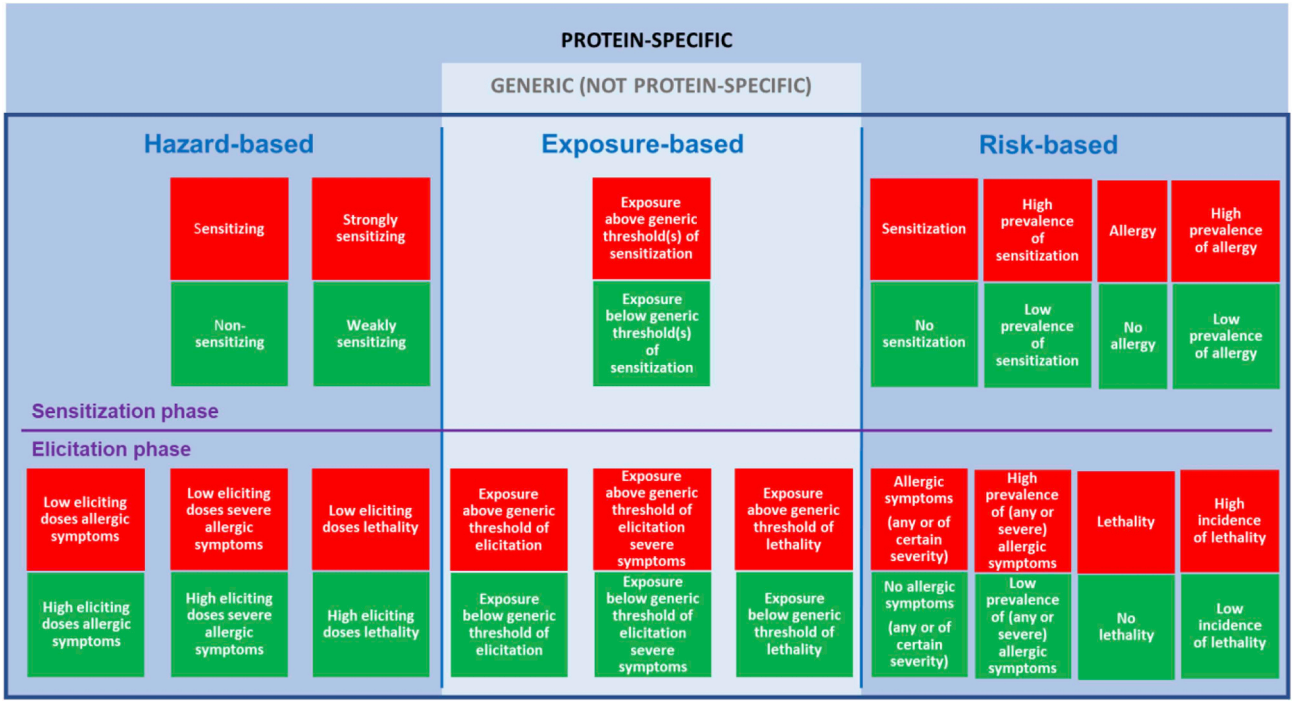

Fig. 3. Overview of (theoretically) possible parameters (red and green boxes read horizontally across) and criteria (red versus green box) for risk management decision-making with respect to IgE-mediated allergenicity of new or modified food proteins. Risk management decision-making could be based on a single parameter/criterion or on combinations of parameters/criteria. Green: an acceptable situation; red: a non-acceptable situation. Each (theoretically) possible option has specific implications for risk management and the methods and data needed for the assessment, which are addressed in Table 1.

with existing allergens as well as to evaluating hazards and risks resulting from *de novo* allergenicity of new food protein products. For assessing IgE-mediated cross-reactivity with existing allergens, adequate test approaches are already available. For instance, Verhoeckx et al. have described a schematic approach (Verhoeckx et al., 2016), which was successfully applied to assess the cross-allergenicity of mealworm proteins in food allergy patients with an existing shrimp-allergy (Broekman et al., 2015, 2016, 2017a, 2017b; Verhoeckx et al., 2014). The results from these studies allowed expressing the allergy risk resulting from this cross-reactivity in terms of the expected prevalence of food allergy to mealworm in the general population, as well as the expected sensitivity for symptom elicitation of the mealworm-allergic population (i.e. the amount of mealworm protein needed to trigger allergic symptoms).

The previously mentioned ILSI Europe Expert Group also presented an approach for scaling allergy risks of foods relative to each other. Two parameters were used to achieve differentiation: 1) the prevalence of IgE-mediated allergy to known allergenic foods in the general population and 2) the sensitivity of the food allergic population for symptom elicitation. The latter was expressed as the ED50-value, representing the dose predicted to elicit an allergic reaction in 50% of the at risk/susceptible allergic subjects, which was derived from the minimum Eliciting Dose (ED) distribution in the allergic population. The authors presented a figure, in which prevalence and ED50 data for several allergenic foods were plotted together as a proof of concept for this scaling approach (Houben et al., 2016). Results from several studies by Broekman et al. (see above) were applied in this scaling approach, from which it could be concluded that mealworm is slightly more potent than shrimp in eliciting allergic symptoms (lower ED50), but is less potent than several other major allergenic foods, while the prevalence of allergy to mealworm may be expected to be comparable to the prevalence of allergy to several regulated allergenic foods (see Fig. 4; and (Hustinx-Broekman, 2017). Such comparison could help regulators and risk managers to put the potential public health risks of a new food in perspective. For instance, proteins or foods with an expected high prevalence of allergy and/or low ED50 value might be categorized as relatively potent allergenic proteins or foods, inducing regulators or risk managers to decide that exposure should be prevented or limited. Conversely, exposure to proteins or foods with an expected low prevalence of allergy and high ED50 may be considered to present an acceptably low risk that does not require specific management. In principle, the scaling approach could also be applied in assisting regulators and risk managers in decision-making with respect to *de novo* allergenicity of new or modified food proteins if data or methods were available to estimate or predict the (expected) prevalence of allergy and ED50-values. Yet it is important to remember that currently methods to predict and assess the hazards and risks resulting from *de novo* IgE-mediated allergenicity of new or modified food proteins prior to market launch are generally lacking.

Each choice of parameter and criterion for risk management decision-making has its specific implications for risk management. Furthermore, some parameters in the overview (Fig. 3) are dependent on other parameters. For example, the prevalence of allergy is likely to depend partly on the prevalence of sensitization, and the incidence of allergic symptoms will depend on a combination of exposure, minimum eliciting dose and prevalence of the allergy. Therefore, the assessment or prediction of some complex parameters require more underlying data than the assessment of other, more straightforward parameters. Consequently, each choice of parameter and criterion for risk management decision-making also has its own set of implications in terms of the associated assessment methods and the data needed for the assessment (i.e. the applicable existing datasets or test methods and study

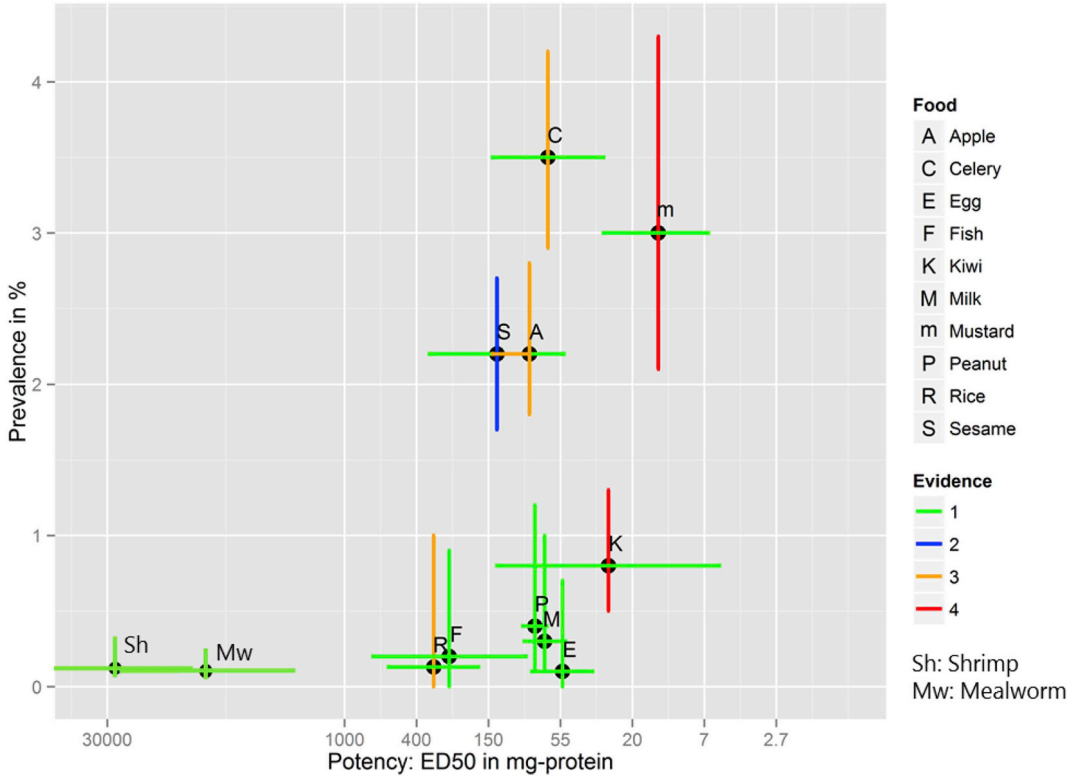

Fig. 4. The cross-reactivity allergy risk of mealworm, a potential new food protein source, is shown relative to that of known major allergenic foods, plotted in a **proof of concept** illustration showing the scaling of allergenic foods according to their public health relevance, based on two, possibly independent parameters: the prevalence of IgE-mediated allergy to the foods and the sensitivity of the food allergic population for symptom elicitation (Evidence = quality level of evidence: 1 = highest, 4 = lowest quality, (van Bilsen et al., 2011); Figure adapted from (Houben et al., 2016) and reproduced with permission from (Hustinx-Broekman, 2017); For further explanation of the figure, parameters and legends, see also (Houben et al., 2016).

approaches needed to generate new data). The next paragraphs will address implications of the different parameters and criteria for risk management and methods and data.

5. Implications of the various optional parameters and criteria

Basing risk management decisions on parameters and criteria derived from hazard alone in the sensitization phase section of the provided overview of possible parameters and criteria for risk management decision-making (top left in Fig. 3) would in principle provide the most extensive consumer protection, but may be over-protective in practice. The most cautious criteria, "sensitizing/non-sensitizing", implies that we would only accept new food protein products that do not have any sensitizing potential. This would mean that every new food would need to be safer than any known food, as absolute non-allergenicity has not been proven for any existing protein-containing food. It is also questionable whether non-sensitizing food proteins do exist at all, and therefore, this parameter/criterion would thus likely appear too conservative. The other extreme can be found in the risk-based elicitation phase section of the overview (far bottom right in Fig. 3), where we would accept any new food protein product as long as no lethal allergic incidents occur, or even at the cost of a few lethal incidents. This would imply that we might accept a situation where a relatively high proportion of the population could become allergic to a product and frequently experience severe allergic reactions as long as these are not or only incidentally lethal. It is questionable whether that would be an acceptable situation for a new dietary protein source, although we should realize that such situation is currently accepted for peanut, nuts, milk, egg, and several other existing foods. Nevertheless, it is likely that parameters and criteria that are somewhere in between those two extremes will be more appropriate for risk management decision-making with regard to new or modified food proteins and may strike the right balance in providing novel food proteins with an appropriate assurance of safety. However, all parameters and criteria have advantages and disadvantages, with each option entailing distinct implications with respect to risk management. Table 1 summarizes implications for risk management for several options according to the suggested parameters and criteria for risk management decision-making.

As indicated before, besides implications for risk management, each option for parameters and criteria for risk management decisions also has implications regarding the associated risk assessment methods and the data needed for the assessment. As we move across the table to the right and down, data requirements in general increase for each parameter. In the case of existing foods or cross-reactivity of new protein products with existing food allergens, the necessary data may be available or could be generated, as shown in the case study on mealworm proteins described above. However, for the assessment or prediction of hazards and risk resulting from *de novo* allergenicity of new food products prior to the intended (broad) market launch, practicable and effective methods are currently largely lacking and can at best be expected to become gradually available in future. Nevertheless, for guiding method development, it is important to consider the advantages and disadvantages of the presented optional parameters and criteria and their implications for method development. In Table 1, an overview of implications for method development is also given. As methods to assess IgE-mediated cross-reactivity are largely available, method development is particularly relevant for the assessment of hazards and risks of *de novo* allergenicity. Therefore, although many implications listed in the table may also apply to cross-reactivity, the implications addressed in Table 1 are mainly focused on those with respect to the assessment of *de novo* IgE-mediated allergenicity.

6. Examples of current practices in risk management decision-making with regard to potential allergenicity of new food proteins or new applications of proteins in food

Several assessments of the allergenicity of new food proteins or new applications of proteins in food have been performed so far that have been used for risk management decision-making in connection with applications to place the respective products on the EU market. In most, if not all of these cases, one or more of the criteria listed in the previous paragraphs have explicitly or implicitly been used. In this paragraph, three novel food applications that have been assessed by EFSA are discussed, along with the criteria applied and the decisions made, with the aim to illustrate how information can be interpreted in the context of Fig. 3 and Table 1. The authors note that since the assessment of the allergenicity of these novel food applications by EFSA, EFSA published a Scientific Opinion (EFSA Panel on Dietetic Products, 2016) (Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283) which provides some instructions/suggestions how to handle allergenicity (section 2.11). This guidance however does not specify the criteria upon which any decision(s) will be made or their relative importance.

Case 1. Chia (Salvia hispanica L.) seed and ground whole chia seed for use in bread (EFSA, 2005, 2009)

The criterion ultimately used for the risk management decision taken in this case was "no allergic symptoms/allergic symptoms" expected. This concerns a risk-based decision applied to the assessment and management of risks in the elicitation phase. EFSA reported that there was no evidence in the literature for any allergic or cross-allergic response after consumption. A safe exposure level excluding allergic reactions (and other adverse effects) was estimated based on the current exposure patterns in non-EU populations. This exposure information was compared to the anticipated intake of chia after market introduction in Europe that had been estimated in the submitted novel food ingredient application. Based on this, the EFSA Panel concluded that the use of Chia seeds in bread with a maximum content of 5% would likely not have an adverse effect (including allergenicity) on public health.

The criteria "non-sensitizing/sensitizing" or "weakly sensitizing/strongly sensitizing" as described for the hazard-based approach in the sensitization phase were not used. The Panel mentioned that the potential sensitizing activity of chia proteins (*de novo* sensitizing capacity) had not been addressed in the evaluation due to the lack of information, implying that a potential sensitizing activity was not excluded.

Data on possible cross-reactivities were, however, included in the assessment:

- Sporadically, allergic cross-reactivity of common crops used for food that are taxonomically closely related to chia has been observed.
- One case study of a patient reacting to oregano and thyme, belonging to the family of *Labiatae* to which also chia belongs, has been published.
- The applicant performed studies on cross-reactivity using a panel of 30 sera from food allergic patients (peanut, tree nuts).

The Panel concluded that there are still uncertainties with regard to the potential allergenicity of chia. However, the Panel recognized the difficulty of predicting the potential allergenicity of this novel food (chia) using currently available methodologies. They noted that the provided complementary information showed no indication of noticeable allergenicity of chia and considered that concerns on this issue could be reasonably addressed by appropriate management measures. Thus, as indicated above, the risk-based approach for decision-making was chosen, based on an anticipated exposure level below the (unknown) threshold of allergic reactions. The decision implied that based on the limited data provided it could not be excluded that the new food

Table 1

Options for parameters and criteria for risk management decision-making regarding IgE-mediated allergenicity of new food proteins and protein sources and implications for risk management and method development. A: parameters and criteria applying to the sensitization phase; B: parameters and criteria applying to the elicitation phase; * Implications for risk management in general are comparable for hazards and risks resulting from cross-reactivity and those resulting from *de novo* allergenicity. In contrast, implications for method development differ. As methods for assessing cross-reactivity are largely available, the described implications for method development are largely focused on those relevant for the assessment of *de novo* IgE-mediated allergenicity; ** with possible distinctions, for instance between proteins in general or strongly sensitizing proteins; *** with possible distinctions, for instance between any effects, severe effects and lethality; **** depending on choice of possible distinctions (see note ***).

A

Options for parameters and criteria for risk management decision making							
Creterion	Hazard-based		Exposure-based	Risk-based			
Accepted	Non-sensitizing	Weakly sensitizing	Exposure below generic threshold(s) of sensitization**	No sensitization expected	Low prevalence of sensitization expected	No allergy expected	Low prevalence of allergy expected
Rejected	Sensitizing	Strongly sensitizing	Exposure above generic threshold(s) of sensitization**	Sensitization expected	High prevalence of sensitization expected	Allergy expected	High prevalence of allergy expected
Implications of optionfor risk management	Likely not a discriminative criterion, as it is questionable whether fully non-sensitizing food proteins do exist. Criterion would probably imply that every new food would need to be safer than current (major) foods.	Criterion would imply the need for defining the parameters/measures and cut-off values for the differentiation between weak and strong sensitizers. However, same levels of IgE do not always imply same health relevance. Therefore, cut-off values may need to be differentiated between proteins or foods or cut-off values would provide different levels of protection for different proteins or	Criterion would require assurance that exposure can be managed and kept below the applicable threshold.	The absence of something and thus also of (future) sensitization cannot be proven. Criterion would probably imply that every new food would need to be safer than current (major) foods.	Definition and agreement on the accepted prevalence of sensitization would be needed.	Criterion would imply acceptance of people to become sensitized. Scientific criteria for identifying allergenic foods of public health importance (ILSI publications) could be applied. However, the absence of something and thus also of (future) food allergy cannot be proven. Criterion would probably imply that every new food would need to be safer than current (major) foods.	Criterion would imply the acceptance of people to become sensitized. Definition and agreement on an accepted prevalence of food allergy for new food proteins and protein sources would be needed.
Implications of option for method development *	If fully non-sensitizing food proteins do exist, validated methods for establishing whether or not a protein (product) is a sensitizer are presently not available and would need development and validation. This would possibly also require definition of what is a sensitizer and of cut-off values.	Validated methods for establishing whether or not a protein (product) is a strong sensitizer are currently not available and would need development and validation.	Generic thresholds of sensitization would need to be elaborated and methods would be needed to assess/predict and monitor exposure.	Methods would be needed that provide measures that predict whether or not sensitization is to be expected. These would possibly need to cover the overall outcome of hazard, exposure and other influential factors.	Methods would be needed that provide measures that predict the prevalence of sensitization to be expected. These would possibly need to cover the overall outcome of hazard, exposure and other influential factors.	Methods would be needed that provide measures that predict whether or not food allergy is to be expected. These would possibly need to cover the overall outcome of hazard, exposure and other influential factors.	Methods would be needed that provide measures that predict the prevalence of food allergy to be expected. These would possibly need to cover the overall outcome of hazard, exposure and other influential factors.

B

Options for parameters and criteria for risk management decision making									
Criterion		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	No severe allergic symptoms expected	Low incidence of severe allergic symptoms expected	No lethality expected	Low incidence of lethality expected
Rejected		Low eliciting dose***	Exposure above generic threshold(s) of elicitation***	Allergic symptoms expected	High incidence of allergic symptoms expected	Severe allergic symptoms expected	High incidence of severe allergic symptoms expected	Lethality expected	High incidence of lethality expected
Implications of optionfor risk management	Acceptance of incidence of: sensitization	X	X	X	X	X	X	X	X
	allergy	X	X	X	X	X	X	X	X
	mild symptoms		X****		X	X	X	X	X
	severe symptoms		X****		X		X	X	X
	lethality				X				X
	Requirement for Risk Management	Definition of and agreement on cut-off values between high and low eliciting dose and the type of allergic symptoms to cover needed.	Assurance that exposure can be managed and kept below the applicable threshold.		Definition and agreement on an accepted incidence of allergic symptoms.	Agreement on and a definition of what we consider severe symptoms.	Agreement on and a definition of what we consider severe symptoms and definition, agreement and acceptance of a certain low incidence of severe		Definition and agreement on an accepted incidence of lethality.
Implications of option for method development *		Methods would be needed that provide measures that predict eliciting doses for (any or specific types of) food allergy symptoms.	Generic thresholds of effect elicitation need to be elaborated. This may be feasible on the basis of threshold data nowadays available. Methods needed to predict and monitor exposure.	Methods would be needed that predict whether or not allergic symptoms are to be expected.	Methods would be needed that predict the incidence of allergic symptoms to be expected. These would need to cover the prevalence of allergy to be expected.	Methods would be needed that predict whether severe allergic symptoms are to be expected.	Methods would be needed that predict the incidence of severe allergic symptoms to be expected. These would need to cover the prevalence of allergy to be expected	Methods would be needed that predict whether or not lethal allergic effects are to be expected.	Methods would be needed that predict the incidence of lethal allergic effects to be expected. These would need to cover the prevalence of allergy to be expected.
				The methods would (additionally) need to cover the sensitivity distribution in the allergic population, the possible exposure patterns and other possible influential factors.		The methods would (additionally) need to cover the sensitivity distribution for severe symptoms in the allergic population, the possible exposure patterns and other possible influential factors.		The methods would (additionally) need to cover the sensitivity distribution for lethal effects in the allergic population, the possible exposure patterns and other possible influential factors.	

protein source might have a sensitizing capacity and might cause food allergy (i.e., the condition; at the expected levels of exposure, not the symptoms).

Case 2. Rapeseed (Brassica napus) protein isolate (Commission, 2014; EFSA, 2013)

In their assessment of rapeseed protein isolate as a novel food ingredient, EFSA paid special attention to the potential allergenicity in populations with existing sensitization or allergy to proteins from plants in the *Brassica* family, which includes mustards, crucifers and cabbages. Sensitivity to seeds of *Brassica rapa* and/or *Brassica napus* was found in 11% (206/1887) of atopic Finnish children with suspected food allergies who were screened using skin prick tests. A subsequent challenge test confirmed that 89% of the sensitized children were allergic to turnip (Brassica rapa). In another study by the same authors, a group of homologous proteins, 2S albumins or napins, were identified as possible food allergens. The authors considered that even the smallest quantity of protein residues in refined or cold-pressed rapeseed oils might be sufficient to induce sensitization. Additionally, there are indications of cross-reactivity between rapeseed and several other foods (studies summarized in the EFSA report) and the EFSA Panel considered it likely that rapeseed can trigger allergic reactions in consumers with mustard allergy. The intake assessment for Rapeseed Protein Isolate that was submitted with the novel food application was, however, not used in the assessment of the allergenicity (sensitization or elicitation of food allergic symptoms).

The EFSA Panel concluded that the risk of sensitization to rapeseed, as well as the risk of cross-reactivity in subjects allergic to mustard, cannot be excluded. The authorisation to place rapeseed protein isolate as a novel food ingredient on the market was given under the condition that food containing this ingredient had to be clearly labelled, allowing people with mustard allergy to avoid the consumption of those products.

In this case, the risk-management decision was made with the intention to assure that allergic consumers would be able to avoid foods containing the novel ingredient, thus applying the criterion “no allergic symptoms/allergic symptoms” expected by exposure management. This also concerns a risk-based decision applied to the assessment and management of risks in the elicitation phase. The approach may properly protect mustard allergic consumers from allergic symptoms to the novel food ingredient due to its cross-reactivity with mustard. Remarkedly however, though a (*de novo*) sensitizing capacity of the novel food ingredient was not excluded and is to be expected, this was not further mentioned/taken into account in the decision.

Case 3. Ice structuring protein (ISP) (EFSA, 2008)

The assessment of allergenicity starts by the Panel mentioning that no method currently exists which can give assurance that a protein lacks the ability to induce an allergic reaction or sensitize an individual consumer to subsequent challenge. The applicant described a number of tests that were intended to generate a body of evidence showing that ISP is unlikely to induce or elicit allergic reactions. Neither sensitization nor allergy was reported (either based on community reports or from an intervention study in volunteers). Additionally, the history of human consumption of fish containing this protein was considered. Furthermore, at the time of application, ISP had already been used as an ingredient in edible ices in the USA and Australia/New Zealand for some time, with no indication of adverse effects. Summarizing all supporting data, the applicant suggested that the proposed ISP preparation is highly unlikely to provoke an allergic reaction in consumers, who are already sensitized to fish, and is unlikely to sensitize potentially susceptible individuals in the general population. The Panel agreed that the results presented by the applicant support these conclusions. Based on the submitted intake assessment for overall toxicity (implicitly including allergenicity) the Panel concluded that the use of the ISP type III HPLC 12 preparation at a maximum level equivalent to 0.01% in edible ices is safe, subject to adherence to the specifications and production practices described by the applicant.

The decision was apparently based on the expected absence of sensitization and allergy (i.e. the condition) upon exposure anticipated from the intended application of the novel food. This is congruent with the criteria of a risk-based decision, yet in this case applied to the assessment and management of risks in the sensitization phase. The criteria applied were presumably “no sensitization/sensitization” expected and “no food allergy/food allergy” expected. Although with these criteria, also the elicitation of allergic symptoms is assumed to be prevented, this criterion was not actually applied, as this would have to be based on a comparison of the level of exposure with the no or minimal effect eliciting doses in an allergic population, which was not the case.

As emphasized by the ESFA Panel in their evaluation, there are no methods that are able to fully exclude a potential sensitizing capacity of a protein. Consequently, although there are no apparent indications that ISP could be a sensitizer, a sensitizing potential cannot be completely excluded. Considering the fact that a sensitizing potential cannot be excluded, in combination with the fact that there are no criteria to distinguish weak from strong sensitizers, it appears that the possible existence of a hazard of any strength was accepted by the EFSA Panel in their risk assessment of ISP.

7. Discussion and future perspectives

In Fig. 3 and Table 1, possible criteria for risk management decision-making regarding the acceptability of new protein products in view of their potential allergenicity are given. In the previous section, three applications relating to novel protein food products and the respective EFSA risk assessments were analyzed in this perspective. From these case studies, it can be noted that even for a protein without any known history of sensitization or elicitation of allergy symptoms, a potential for causing sensitization and allergy was not excluded. This seems reasonable from a methodological point of view (the absence of something cannot be proven), but also when considering that the existence of fully non-sensitizing or non-allergenic (food) proteins is questionable. It is therefore unlikely that a qualitative hazard-based criterion such as “non-sensitizing/sensitizing” can effectively be used, or that methods allowing to verify this criterion will be achievable. Quantitative hazard-based criteria (“weakly sensitizing/strongly sensitizing” or “low eliciting doses/high eliciting doses”) may be feasible to implement, but realization would depend on consensus on the establishment of a cut-off value to discriminate between weak/strong sensitization potential and high/low elicitation doses. These criteria, however, are preferably not used individually but in combination with the expected level of exposure, which is illustrated by the three case studies described in the previous paragraph: in the novel food applications for chia, rapeseed protein isolate and ice structuring protein it was argued that sensitization, allergy or elicitation of allergic symptoms would not be expected at the anticipated levels of exposure or after implementation of appropriate risk management measures. In all cases, the proven or potential existence of an evident hazard was accepted and a risk-based decision was made, aiming to reduce the potential risk to a zero- or minimal level. There was no explicit acceptance of a certain level of risk (a definite type or frequency of sensitization, allergy development or elicitation of allergic symptoms).

In view of the intrinsic difficulty of society at large in dealing with and accepting risks, the preference to aim at zero-risk may be understandable, even though most stakeholders know that zero-risk will not be feasible in many circumstances. If future research underpinned the existence of a Thresholds of Allergenic Concern and allowed the establishment of generic exposure thresholds for sensitization and effect elicitation, criteria based on these could become feasible. However, we should realize that criteria aspiring to minimize risks might interfere with innovations in food supply and restrict food choices. For instance, the improvement of the future viability of our food supply through the

introduction of new sustainable protein sources will be associated with higher intake levels of novel proteins, which rather likely will lead to risks of sensitization and allergenicity. A similar situation exists for the risk management of existing allergenic foods, for which it is evident and accepted that a zero-risk approach is not feasible (Madsen et al., 2012). In such cases, Other Legitimate Factors may be considered in the risk management decision-making process and may force (or enable) us to move away from decisions aiming at zero-risk towards decisions accepting a certain type or frequency of adverse events. Acknowledgement of the realities in the risk assessment and management of existing allergenic foods resulted in the development of a quantitative guidance for precautionary allergen labeling based on accepted residual risk levels (Allen et al., 2014; Allergen Bureau of, 2012; Taylor et al., 2014). However, we should realize that setting accepted residual risk levels is a risk management responsibility and that these levels are not yet formally approved by regulatory bodies or European risk managers. To the best of our knowledge, such an approach has not been applied to novel protein applications yet. From the case studies it is further evident that the hazard and risk assessment outcomes not only are used for deciding on the approval of a new product, but also to inform decision making regarding risk management measures (e.g. approval for maximal levels in foods or with the provision of adequate labeling).

In Table 1, the type of data and methods needed for applying the possible criteria are addressed. Most criteria depend on the availability of substantial amounts of product/protein-specific data. Advances in allergen risk assessment methods and a large quantity of data generated throughout the last decades (Crevel et al., 2014; Klein Entink et al., 2014; Kruizinga et al., 2008; Madsen et al., 2009; Remington et al., 2015; Spanjersberg et al., 2007, 2010) make the application of some criteria feasible already now, or within the coming years. This applies particularly to situations, where already sensitized or allergic individuals can be identified, or to the assessment of risks due to cross-reactivity, as was demonstrated for insect proteins. However, in the absence of relevant data, which is particularly critical for the assessment of possible *de novo* sensitization and allergenicity of new food proteins, methods to generate product/protein-specific data in line with most of the criteria mentioned in Fig. 3 still are largely lacking or lack predictability or validation. The ImpARAS network has put much effort into definition and characterisation of general requirements for the development and validation of such methods. We presume that even the development of the concept of Thresholds of Allergenic Concern (TAC) could be feasible if it was based on threshold data accumulated during the past decade. As mentioned earlier, this idea is currently being explored as an activity from the ImpARAS team.

Future research may provide more insights into why some proteins are more allergenic than others, and may increase the possibilities for quantitative risk assessment. Particularly, the introduction of new protein sources to improve the sustainability of our food protein supply will lead to consumption at high, nutritionally relevant intake levels, and risk assessment approaches aiming at zero-risk will not work. Certain levels of risk will have to be accepted. Projecting the potential allergenic risks of new protein products against a benchmark developed on the basis of the known allergenicity of existing foods, as was done for mealworm proteins as described in this paper, will help to determine relative risks in comparison to existing foods and support regulators in decision-making. Better knowledge on why some proteins are more allergenic than others could be a starting point for the development of assays that can predict the allergenic potential of new or modified proteins and allow the assessment of *de novo* allergenicity relative to existing foods. State-of-the-art technologies and recent advances have been addressed in several publications from the ImpARAS network (Bogh et al., 2016; Epstein and Verhoeckx, 2015; Mazzucchelli et al., 2018; van Bilsen et al., 2017), while new methods, e.g. based on machine learning (Westerhout et al., 2019 submitted), are being explored. Yet, a clear outline of preferred decision-making criteria is needed from the risk management sector to help guide research during method development and ensure the applicability of newly developed methods for the questions at hand.

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