	Event	GMOWorkshop2021									
N°	How can available human data on sensitisation and elicitation of allergic reactions to known allergens be used to improve the risk assessment of innovative proteins?	What is the usefulness of the current FASTA algorithm and what alternative approaches could be used?	Are current allergen databases (e.g., Allergenonline, Allergome, Allermatch, Compare, WHO/IUS) effective for allergenicity assessment and how can they be improved to better support the risk assessment?	What are the most relevant test materials to predict allergenicity, e.g. individual proteins, extracts (raw or processed), whole food matrix or a combination of all these?	What molecular stability parameters (e.g., effect of processing, enzyme degradation studies, temperature, pH) or other physicochemical properties (e.g., glycation, aggregation, pressure stability,) should be considered in the allergenicity risk assessment?	Human sera (IgE binding) are used for an allergenicity assessment in two instances, i) when the source of the transgene comes from an allergenic food, and ii) when a hit is identified following bioinformatic analysis. There are issues related to availability and clinical characterisation, number of serum samples needed, interpretation of the results and how to assess minor vs major allergens. When and how should human sera or alternatives be used to address these challenges?	What are the most effective methods to predict allergenicity (e.g., sensitisation, elicitation, or both), considering that the goal of the risk assessment is to prevent harm?	When and how should in silico and in vitro assays be done to better support allergenicity prediction, e.g., used always or only when certain other predictive tools have exceeded a threshold (e.g., 35% similarity with an allergen, resistance of a protein to pepsin over X minutes), and how should they be integrated?	How best to validate in silico and in vitro methods with animal models, ex-vivo models and others?	How can post-market monitoring tools be developed to address the uncertainties in pre-market allergenicity risk assessment?	How to determine acceptable levels and/or thresholds of protein that will not raise safety concerns about cross-reactive or de novo allergic responses?
1	First the data would need to be collected and made accessible. Is that already available?		There are indeed various allergen databases (DBs) available, some with distinct purposes and thus with varying inclusion criteria and not all are maintained regularly. Reviews of these various DBs are available (Radauer, 2017; Radauer & Breiteneder, 2019; Sircar et al., 2014), in which the features and specific purposes of each resource are described. I would be interested in asking a different question: how can stakeholders (at large) facilitate the implementation of improvements (to better support the risk assessment) in a sustainable way? I will relay here the experience from the COMPARE DB. Each year, the program									The question of thresholds is a delicate one, considering that there is such a wide range of degrees to which allergic patients can demonstrate symptoms/reactions - one threshold could be safe for some patients but not for others. I would suggest involving clinicians (practicing allergy doctors) in this discussion.
2	When available these data can be used to developed better predicting in silico tools. Available clinical data should be integrated in big databases to develop machine-learning models, thus facilitating/improving the risk assessment of novel proteins.	FASTA is similar to BLAST algorithm, both using heuristics to perform fast local alignment searches. Both algorithms can be applied to search similarities among protein and nucleotide sequences.	Different databases gather pertinent information on each allergen, which in most cases are excellent tools for rapid consultation. Some databases are more complete and updated than others, normally providing distinct or complementary information among them. However, at this point, they are still not very effective for allergenicity assessment considering that the information present for each allergen is often incomplete and not linked to other databases. Data on primary sequences of most know allergens are already available, but conformational structures experimentally determined and made available for	Every test material can contribute with distinct data in terms of "predicting" potential allergenicity and its importance depends on the stage of knowledge on the allergenic potential of a specific food. Extracts from raw or processed foods are the most commonly used test materials, being a good option to evaluate the allergenic potential of a specific food. However, these extracts often do not reflect the actual allergen content of the whole food, considering the nature of different allergen (pH, polarity, hydrophobicity, etc). Therefore, a combination of all test materials would probably provide more a realistic assessment of the allergenic	In principle, all parameters could be considered as relevant in the allergenicity risk assessment but at different degrees. With the data available so far and depending on the allergen or group of allergens, the impact of distinct parameters on protein allergenicity is quite different and still uncertain. For instance, parameters such as glycation and aggregation have low or inconclusive impact on the IgE-binding capacity of most allergens, irrespectively of their source (animal or plant). Therefore, glycation and aggregation should have "lower weight" than other parameters (e.g changes in protein fragmentation induced by enzyme degradation) in	Human sera and often-other biological material (cells) are much needed to address the challenges related to allergenicity. Of course, the type of material is highly variable depending on several factors associated with patients, which retrains the interpretation of the results. However, these problems are incremented by the fact that there are no standardized protocols for methods using these biological materials. For instance, protocols from performing in vitro digestion in a concerted away have advanced, which facilitates comparing the results obtained by different groups but using the same protocol. The same concept must be	Sensitization is not always followed by elicitation event, but methods determining elicitation are the best correlated with allergenicity, since those methods are the best way to conclude if a protein is able to induce an allergic response in patients. However, methods determining what makes a protein to be allergenic in the first place would be more relevant to predict potential allergenicity of novel food proteins. Ultimately, both should be considered as relevant for allergenicity prediction, although with probable different weights.	In silico assays are a good starting point to determine if a protein may or not be an allergenic candidate. Therefore, when a protein is entirely new or from a novel food source, the in silico tools may be used to determine its allergenic potential. However, it is important to determine if these in silico tools are good predictors for the proteins already well established as allergens. If these tools are able to accurately predict the allergenic potential of a known protein, they will probably be helpful in determining the allergenicity of a new protein. For instance, if a new protein share high similarity and potential epitopic regions with a known allergen, this new protein have high	The validation of in silico and in vitro assays would benefit from multiple data. At this point animal food allergy models are gaining ground and importance but their correlation to what happens in in vivo (patients) is still quite unknown. Validation would always require in vivo (patient) testing, as least until data can support a good % of certainty regarding the predicting capacity of in silico and in vitro assays.	-	At this point, threshold levels of an allergen that will not raise safety concerns are still being determined at individual level, which are being extrapolated to population level using mathematical models. Over the last years, a great work has been performed to convert individual responses to populational ones, but this can only be done with increasing level of confidence following the rhythm of novel data from clinical studies. This type of work is being carried out for well know allergenic foods (still with very limited information on several well-known allergenic foods), which means that we are still far from having absolute knowledge on cross-reactive and de novo
3											
4	Very difficult task because the risk assessment is a predictive approach and the risk associated to innovative proteins is predicted according to the weight of evidence approach (comparison with known allergens or toxins).	Usefulness for predicting the relationships between proteins (allergens, toxins) but what is of paramount importance is the degree of similarity between proteins (coverage of the complete sequence, not of a short stretch of sequence).	This is a important goal to use confident allergen database, that means databases containing carefully selected allergens on the basis of their real (not suspected) allergenic potential. In most of the allergen database, one can find some so-called allergens whose the allergenic potential is dubious.	The effect of food matrix seems of paramount importance but remains difficult to be taken into account in in vitro experiments (what food matrix to use to sufficiently mimic the physiological conditions?).	The stability parameters simply offer some prediction about the possible resistance the potential allergens might display in physiological conditions. In my opinion, the enzyme degradation studies and the resistance to heat denaturation, behave as a sort of 'scratch-tests' that allow to predict the ultimate resistance of allergens to very drastic environmental conditions. In turn, it is conceivable that allergens able to resist such drastic conditions, will be able to resist mild physiological conditions (digestive enzymes, industrial process) and can display some allergenic potential in physiological conditions.	The use of sera samples from allergic people is an important tool. However, the occurrence in the sera of IgE directed against the hit identified by bioinformatic analyses must be assessed before performing the analysis. If the identified hit corresponds to a minor allergen, the use of IgE-containing sera from allergic people is less interesting.	Both.	The threshold of 35% identity used in alignment comparisons, suggests that the protein expressed by a PGM exhibits an overall folding which superposes to the folding of a genuine allergen (rmsd close to 1-1.5-A). A careful inspection of the sequence alignment is necessary to better define the extent of the identity: covering the entire sequence or a part of the sequence? If possible, a molecular modeling can be performed to assess the hit exhibits an overall 3D-conformation similar to that of the genuine allergen. When the 3D-model becomes available, one can easily delineate the putative cleavage sites for pepsin and trypsin	I don't know.		This is the still poorly-know question of the allergen threshold necessary to sensitize or trigger an allergic response in sensitized people. Very difficult to answer this question!

5	The sensitisation (IgE) pattern at the protein level (or even epitope level) can inform on the families of proteins inhering allergenic potential. Further, it can provide information on physicochemical properties of conferring the proteins allergenic potential.	I believe it can be a useful tool. However, we will need to know the degree on homology that provide a risk for cross-reactivity. Also more information on the importance of the primary structure compared to secondary, tertiary and quaternary structure is needed. In silico analyses should be performed in the light of the abundancy of the individual proteins.	Some databases are better than others. The amount of data available in each database differs. It is our experience that results from homology searches does not always correspond to cross-reactivity.	The usefulness of the individual materials may depend on the exact analyses performed, so it is a difficult question. In the best of all worlds proteins extracts together with whole food should be assessed. Extraction processes as well as protein purification may impact on the proteins themselves.	The stability parameters could in general provide information on allergenicity, however, as no characteristics of allergens have been identified, we should be careful making definitive conclusions on the outcome.		No single method can provide a definitive answer, so an array us methods would provide the most solid predictive results.		Perform an arrays of in silico and in vitro methods and compare them with an array of in vivo and ex vivo methods. Make sure to include a range on non-allergenic, low-allergenic and high-allergenic proteins/foods.		We do not know the dose-response relationship for de novo sensitisation, so at the moment it will be difficult to determine acceptable levels of proteins to prevent sensitisation.
6				To evaluate relative digestibility of proteins, studies indicated that purified proteins derived from a heterologous expression system are suitable materials in most cases. In some cases when it is difficult or not possible to express, purify or get the protein (e.g., membrane protein) in an appropriate buffer, the protein extract from plant tissue would be a more appropriate material. Therefore, there should be some flexibility and the proper test materials should be determined according the situation. Doing digestion in complex food matrix can make it very difficult to interpret the data without adding additional value to the assessment.	These molecular stability data would not add additional values to allergeneicity risk assessment based on what we know. Enzyme degradation studies may continue to provide some value based on the weight of evidence if used properly.	Even when a protein is from an allergenic food source, it doesn't mean that protein per se is an allergen. Human sera may add value only when a hit is identified with bioinformatic analysis.		In silico analysis should be always done and play a primary role. In vitro analysis should be done only when there is a concern from in silico analysis.	Start with known allergens based on the knowledge from the scientific studies. Additional research will also be needed.		Based on the knowledge from scientific studies. Additional research will also be needed.
7	Available human data on sensitisation and elicitation are extremely important in order to settle the prevalence of food allergies but also to understand the clinical relevance of known food allergens. There is a huge number of food proteins capable of inducing an allergic reaction, each one of them characterised by provoking very different symptoms. In the case of innovative proteins, none clinical data are available to define their risk assessment. However, these "novel" proteins can be homologues to the already well-known allergens, presenting similar aminoacid sequences, structures and even epitopes, which could lead to cross-reactivity phenomena in	--	These databases are useful tools for the first steps of allergenicity assessment. All information regarding sequence, structure, biochemistry, as well as available experimental and human allergenicity data (e.g. case reports, in vitro and in vivo allergenicity studies, cross-reactivity) can contribute to a better understanding on the target allergenic protein and to start implementing a risk assessment. These data are undoubtedly the starting point for the establishment of guidelines to perform all the following protein analysis and human testing necessary for the risk assessment of a novel protein.	In my opinion, all test materials are important to predict allergenicity. First we have to understand the behaviour of the protein itself in order to characterise it and establish an overview on their potential allergenicity and the possible tests that can be performed. After that, it is important to evaluate what happens in a real food, because an allergic individual will react to a protein included in a food, where there are present a lot of compounds which can interfere with the allergenic proteins. Additionally, the evaluation of the processed food is extremely important since it is already well-known that every food processing technique can have an impact on	Proteins are very complex molecules and their allergenicity can be highly affected by several conditions and parameters. There is a lot of available data reporting these effects on the allergenicity of a protein. Food processing is one of the most important parameter since many studies showed the high impact of some processing techniques on food allergens. Food processing can also induce the occurrence of Maillard Reactions (glycation), which can further induce interactions with reducing sugars present in food matrix. Similarly, digestibility play a crucial role on protein stability since gastro-intestinal digestion and digestive enzymes will be responsible for the	Sera from allergic patients are very difficult to obtain because many ethical issues are requested by hospitals to protect the privacy of patients. The use of human sera may also be problematic due to their high variability according to age, gender or the environment surrounding the patient. Different sera from distinct allergic patients can demonstrate variable responses, in particular as diverse patients react to distinct epitopes of each allergen or to different allergens within each specific food. Moreover, the availability of well-defined patients' sera, with a well-documented clinical history is a limiting factor in this	--	In silico and in vitro assays are important to perform in every allergenicity assessment. These approaches rely on bioinformatics/databases and protein chemistry to characterise native and altered food proteins, which may explain why certain food proteins induce sensitisation of the immune system, while others are tolerated. In silico assays performed by itself are not enough to settle a first allergenicity assessment and it is recommended to perform several in vitro tests. These assays such as immunochemical assays (western blot or ELISA) provide information on the molecular weight, stability to heat and the	--	It is important to develop efficient methods for the detection of allergenic proteins at trace amounts in processed foods. By the use of these methods consumers can be aware of the content of the products they are buying, avoiding unwanted adverse reactions. Several strategies are been developed targeting the allergenic protein itself or an indirect marker of the presence of the allergen such as by the use of DNA-based methods. Both strategies revelead to be successful in detecting trace amounts of target allergens. Of course, these methods need to be harmonised and implemented in food industry. Alternatively, portable devices which	--
8	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX case by case	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX
9	What an innovative protein means and whether newly synthesized proteins, as a result of genetic modification - with the known approaches or as a result of genetic editing - are also meant? In principle, human data of reaction to known food allergens could be embedded in risk assessment models	No experience	No experience. Generally user-friendly and informative databases are needed.	It may be a good idea to follow the toxicological testing approach, namely to examine the individual protein to avoid the influence of other ingredients in the food. Food is complex matrix. This will allow the test conditions to be standardized. On the other hand, overwhelming amount clinical data is collected using total food proteins, clinical investigations not using single proteins for dose-response curves.		In any case, it is necessary to have information exchange between competent authorities, hospitals, emergency centers and laboratories, and the can also be used for risk assessment. Based on clinical human data and in vivo animal models (LOAEL and NOAEL) it can be sat a threshold for different susceptible groups, e.g. infants, individuals with compromised immune systems	1.Dose-effect and dose-response relationships assessment; 2. The adverse outcome pathway (AOP) concept can by framework for collecting and organizing information relevant to an adverse outcome at different levels of biological organization- to structure the available in vivo and in vitro mechanistic data for allergic sensitization to food proteins (van Bilsen at all, 2017)		An important issue in silico model to by validated with animal methods. The principles of 3R are also considered. The validation is based of its applicability domain. It can by follow the principles for QSAR validation by the Organisation for Economic Co-operation and Development (OECD, 2004.OECDPrinci:2004) state that in order: "to facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information: a defined endpoint; an unambiguous algorithm; a defined domain of applicability; appropriate measures of goodness-of-fit, robustness and	The monitoring tools can include data from: 1. consumption survey or exposure assessment, 2. product analyses – harmonized analytical methods and measurement units, and 3. clinical human data	

10	The use of human data could help in the construction of probabilistic models for evaluating novel proteins using known data and variables over known ones. In addition, human data could aid the implementation of new, faster and cheaper evaluation technologies (e.g. organs-on-chips and in-silico tools).	The FASTA algorithm is useful when it comes to encoding the primary sequence of a protein for example. Unfortunately, however, it does not provide information about the other structures that are very important for functionality. Therefore, the use of the FASTA sequence is very useful in the search for amino acid similarities but fails to position the latter in space. This leads to the singular inactivity of the FASTA sequence in risk assessment contexts, but other evidence is needed. It would be very useful to unify information of all the structures of a single protein (e.g. FASTA + crystallographic data) in a single file.	Allergen databases contain a lot of information useful for assessing the allergenicity of a given compound (e.g. protein). However, there is no possibility to access the data in a transparent way to draw different conclusions or add evidence to one's hypothesis.	Allergenicity should be conducted on the pure protein in question to appreciate every single possibility of risk. After that, the evaluation should also be carried out also in the product to be marketed - considering any transformations in which the product in question could undergo -, thus highlighting any synergistic or antagonistic effects.	All the physiological parameters of the organism intended for consumption should be considered and the historically known parameters for modifying the allergen (e.g. structurally or chemically) that the product could encounter before reaching the final consumer.	Human and alternative sera should be used when any other evidence does not allow us to make a definitive assessment of the object in question. Interesting is the implementation of in vitro systems such as organ-on-chip. Although regulatory science should legislate to establish a method and validate it that is accepted by the scientific community.	Given the increase in population, the maintenance of food safety and the inexorable increase of compounds discovered to be allergenic, the best method to predict allergenicity turns out to be both sensitization and elicitation to guarantee food safety.	When and how in silico and in vitro tests should be performed to better support allergenicity prediction, for example, always used or only when some other predictive tools have exceeded a threshold (for example, 35% similarity to an allergen, the resistance of a protein to pepsin beyond X minutes), and how should they be supplemented? This is a very peculiar speech because we are in limbo between speeding up the evaluation of food safety and guaranteeing it. - In-silico analyzes as performed by computational systems, in continuous evolution, do not require a lot of time and expenditure of human energy to be performed. The latter	The best method for evaluating models, in general, is to carry out pre-evaluation and evaluation phases in a collaborative system of interested parties to analyze reliability and relevance. The output of these steps, after going through a peer-review system, can be presented to managers for approval at the community level.	Managing a public database internationally in which interested parties can enter and use data would go a long way in analyzing every single facet of the story. However, this also leads to the creation of artificial intelligence able to manage these amounts of data.	Traditional risk assessment using the No Observed Adverse Effect Level (NOAEL) method and uncertainty factors; the BenchMark Dose (BMD) and the Margin of Exposure (MoE) approach; as well as probabilistic models.
11	When product developers select genes/proteins to confer novel desirable traits, protein sequences are evaluated using a search algorithm and an allergen sequence database the goal being identification and discontinuation of potential allergens. Regardless of whether a protein shows sequence similarity to a sensitizer or elicitor, whether injurious through dermal contact, inhalation or ingestion or if allergen status is supported by clinical or only in vitro data, product developers would not pursue such a protein because it may be an allergen. The decision to proceed with a particular protein is "yes/no", there is no mitigating factor	The current application of a FASTA search to identify the relatedness of known allergens to proteins that confer desirable traits is highly effective. An evaluation and validation of thresholds, % identity + window size vs. E-value statistic would be beneficial as it could help to improve the specificity of the search, in particular the false positive rate of the current % identity + window size threshold. It is important to note that it is critical that the alignment be inspected to understand the origin of threshold exceeding alignments. Is/are alignment(s) the product of compositional bias or do they reflect alignment within a specific domain but not	Certain databases are effective for allergenicity assessment while others are not, FASTA search capability through a web portal and a mechanism to permit download of the database in FASTA format are essential. Allergenonline, updated annually by expert panel, permits FASTA searches to be conducted on web portal, no obvious method for a download of the actual sequence database in FASTA format. Allergome, updated regularly, no FASTA search capability or download capability. Allermatch, uncertain update cycle for "AllergenDB", "AllergenDB" is a hybrid of Compare, IUIS and UniProt, FASTA searches to be	Some combination of the listed materials would likely be needed to "predict" allergenicity. A tiered approach would be required where initially, the "prediction" should employ purified protein. In addition, it should be considered whether a validated method has been developed that makes such "prediction" possible; and an understanding has to be established about what is being "predicted", sensitization, elicitation or some other parameter? Raw or processed extracts, whole food matrix or a combination might better mimic actual exposure or consumption scenarios. However,	Several stability parameters and other physicochemical properties (e.g., glycation, lipid binding, phosphorylation, aggregation) were investigated for the most representative plant families of allergens and animal allergens in EU cost action 'ImpARAS'. Certain parameters, like heat stability, resistance to proteolytic activity and structural stability are considered important for protein allergenicity, but clinical data directly linked to these parameters are lacking, showing that no single distinct molecular parameter (or pattern) found within one protein family is exclusively responsible for the	CODEX (2009) recommended a human IgE binding for proteins from allergenic foods or with bioinformatic hits. To achieve a 99-percent certainty that the new protein is not an allergen, a joint FAO/WHO Expert Consultation required a minimum of eight relevant sera for a major allergen and a minimum of 24 relevant sera for a minor allergen, respectively. It is recognized that these quantities of sera may not be available due to the ethical challenges to validate the sera as clinical relevance by using Double-Blind, Placebo-Controlled Food Challenge. For these reasons, GM crops expressing proteins with these two instances will not be	The allergenicity harm is the product of exposure and hazard. From a practical standpoint, GM crops expressing proteins with a realistic allergenic hazard will not be developed. If a protein is isolated from a source known to cause allergy, or if bioinformatic analysis indicates potential cross reactivity with a known allergen, then screening serum from sensitized individuals for IgE reactivity to the introduced protein is a robust way to determine elicitation hazard. Likewise, low concentration of an allergen in food and digestive instability would be predicted to reduce the severity of symptoms in sensitized individuals. The more challenging allergenic risk assessment for a	The weight of evidence approach has been used effectively by EFSA and other global regulatory agencies for GMO risk assessment over last two decades. Collectively, the source of organism, sequence comparison to known allergens, and stability of a new protein to digestive enzymes provide overall information on a newly expressed protein. Bioinformatics analysis is a critical gate keeper, the other end points such as protein stability to heat and digestive enzyme can provide additional useful information on the property of the protein. As described in Question 7, GM crops with proteins exceeding in silico thresholds (>35% similarity with a known allergen) will not be	It is agreed at international level to mainly use methods that have been standardized and validated, and that are specific, robust and able to be predictive of the risk. In silico or in vitro methods with animal models, ex-vivo models and others were proposed for the possible risk assessment of allergenicity. Several available cell-based assays (basophils, Caco2, T84, HT29, M cell) have not been well studied for allergens with proper control proteins in experiment designs. Also, the co-relationship between responses from cell - based assay and in vivo conditions is not well understood. The experimental animal models are often result in highly variable	It is worth emphasizing that since its introduction in the 1990s, no commercialized GM crop has ever been considered an allergenicity risk due to the genetic modification. Research institutes, government agencies and NGOs with sterling reputations have done numerous allergenicity assessments for the GM food in the market. Yet, not one peer-reviewed publication has reported an allergenicity potential. These publications indicate that industrial practice following the current international guidance (e.g. CODEX) minimizes the uncertainties in the pre-market allergenicity risk assessment.	As described in question 7, The allergenicity risk is the product of exposure and hazard. According to the guidelines adopted by the Codex Alimentarius Commission (Codex, 2009), a protein is not likely to be associated with allergenic hazard/exposure if: 1) the protein is from a nonallergenic source, 2) the protein does not share structural similarities to known allergens based on the amino acid sequence, and 3) the protein is rapidly digested in mammalian gastrointestinal systems. Introduced protein levels would not raise any safety concerns if no allergenicity risk was determined. Given thresholds defined in the international or
12	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX	Please refer to responses submitted by XXXXXX All these.	Please refer to responses submitted by XXXXXX This may depend on how the innovative protein is consumed. Test parameters relevant for the protein/food under consideration.	Please refer to responses submitted by XXXXXX Hope to learn more about this from the workshop.	Please refer to responses submitted by XXXXXX Both	Please refer to responses submitted by XXXXXX Is it possible to establish a threshold? How can this be done (in general or on an individual basis)? Hope to learn more about this from the workshop.	Please refer to responses submitted by XXXXXX Cannot answer this question.	Please refer to responses submitted by XXXXXX Developing a possibility of online reporting of complaints or symptoms by doctors and consumers (separate tools).	Please refer to responses submitted by XXXXXX Acceptable levels and/or thresholds can only be determined when information is available of elicitationand sensitisation. Whether an ED01 or ED05 etc is acceptable would be a risk management decision.
13	Information on the innovative (new) proteins is necessary. The 'potency' of the allergenicity of the proteins can be compared to that of known allergens.	I do not know enough about FASTA but assume that all relevant information contributes to a better risk assessment.	In my view, these are helpful databases to better understand the possible allergenicity of a protein/food.								
14	The available human data on sensitisation and elicitation of allergic reactions to known allergens can be used to perform a dose-response curve.	The FASTA algorithm could be used to search for allergenic protein sequence.	Since we don't use the above databases, we cannot provide information for better support the risk assessment.	Combination of all the above materials.	All the above parameters have to be considered in the allergenicity risk assessment.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.	I have no adequate skills in the human clinical field to formulate the answer to this question because I work at the Istituto Zooprofilattico.
15	When product developers select genes/proteins to confer novel desirable traits, protein sequences are evaluated using a search algorithm and an allergen sequence database the goal being identification and discontinuation of potential allergens. Regardless of whether a protein shows sequence similarity to a sensitizer or elicitor, whether injurious through dermal contact, inhalation or ingestion or if allergen status is supported by clinical or only in vitro data, product developers would not pursue such a protein because it may be an allergen. The decision to proceed with a particular protein is yes/no, there is no mitigating factor	The current application of a FASTA search to identify the relatedness of known allergens to proteins that confer desirable traits is highly effective. An evaluation and validation of thresholds, % identity + window size vs. E-value statistic would be beneficial as it could help to improve the specificity of the search, in particular the false positive rate of the current % identity + window size threshold. It is important to note that it is critical that the alignment be inspected to understand the origin of threshold exceeding alignments. Is/are alignment(s) the product of compositional bias or do they reflect alignment within a specific domain but not	Certain databases are effective for allergenicity assessment while others are not, FASTA search capability through a web portal and a mechanism to permit download of the database in FASTA format are essential. Allergenonline, updated annually by expert panel, permits FASTA searches to be conducted on web portal, no obvious method for a download of the actual sequence database in FASTA format Allergome, updated regularly, no FASTA search capability or download capability. Allermatch, uncertain update cycle for "AllergenDB", "AllergenDB" is a hybrid of Compare, IUIS and UniProt, FASTA searches to be			CODEX (2009) recommended a human IgE binding for proteins from allergenic foods or with bioinformatic hits. To achieve a 99-percent certainty that the new protein is not an allergen, a joint FAO/WHO Expert Consultation required a minimum of eight relevant sera for a major allergen and a minimum of 24 relevant sera for a minor allergen, respectively. It is recognized that these quantities of sera may not be available due to the ethical challenges to validate the sera as clinical relevance by using Double-Blind, Placebo-Controlled Food Challenge. For these reasons, GM crops expressing proteins with these two instances will not be	The allergenicity harm is the product of exposure and hazard. From a practical standpoint, GM crops expressing proteins with a realistic allergenic hazard will not be developed. If a protein is isolated from a source known to cause allergy, or if bioinformatic analysis indicates potential cross reactivity with a known allergen, then screening serum from sensitized individuals for IgE reactivity to the introduced protein is a robust way to determine elicitation hazard. Likewise, low concentration of an allergen in food and digestive instability would be predicted to reduce the severity of symptoms in sensitized individuals. The more challenging allergenic risk assessment for a	The weight of evidence approach has been effective for GMO risk assessment over last two decades. Collectively, the source of organism, sequence comparison to known allergens, and stability of a new protein to digestive enzymes provide overall information on a newly expressed protein. Bioinformatics analysis is a critical gate keeper, the other end points such as protein stability to heat and digestive enzyme can provide additional useful information on the property of the protein. As described in Question 7, GM crops with proteins exceeding in silico thresholds (>35% similarity with a known allergen) will not be developed. For some cases, in vitro IgE			As described in question 7, The allergenicity risk is the product of exposure and hazard. According to the guidelines adopted by the Codex Alimentarius Commission (Codex, 2009), a protein is not likely to be associated with allergenic hazard/exposure if: 1) the protein is from a nonallergenic source, 2) the protein does not share structural similarities to known allergens based on the amino acid sequence, and 3) the protein is rapidly digested in mammalian gastrointestinal systems. Introduced protein levels would not raise any safety concerns if no allergenicity risk was determined. Given thresholds defined in

16	The understanding of what genetic predisposition may be prone to allergen proteins will help us learn about what intrinsic characteristics from allergen proteins have the interaction with them.	Yes, it's useful but the criteria are set at very conservative level such that it easily causes false positive results which may be difficult to experimentally test them.	The COMPARE database has the most transparent process for compiling and updating allergen sequences, and shall be set up as the standard. Certain protein sequences were collected based on homology comparison and secondary, indirect clinical data as evidence. For example, allergen A has clinical proof and a protein B is collected if it has over 35% sequence identity and IgE cross-reactivity lab data. However, a third protein C could also be collected in the database if C shares 35% identity with B (but A) and may have IgE cross-reactivity between C and B. In this case, allergenicity of protein C is questionable as it's	Individual proteins are good enough as the model test materials.	They are informative but also lead to inclusive assessment regarding allergenicity.	Only if they become commercially available and easy to access. Otherwise, the ethical approval and difficulties to acquire them make this approach almost impractical or impossible.	So far, none. WoE will continue to be the practical approach	As in the current WoE approach.	at least demonstrate it can be validated.	In link with clinical data. If a class of proteins has a safe use history post-marking and no clinical evidence shows this class of proteins has been ever a source of allergen, then the safety for proteins in the same class shall be granted.	
17	Human data on sensitisation and elicitation of allergic reactions to known allergens is of utmost importance. It is real data, which reflect all possible factors that have an influence on allergenicity. The available human data on sensitisation and elicitation of allergic reactions to known allergens can be used for better modeling of the processes, that happen in both of the phases. The created models can be used as a pipeline representing the route of the protein from the entrance into the body to the place and time, where the protein is defined as an allergen. That pipeline could be included as a part of the weight of evidence approach used in the	The FASTA algorithm allows a search for sequence similarity between a query protein and the known allergens. Sequence similarity appears to be more important for the assessment of cross-reactivity compared to sequence identity. It counts for the presence of gaps and similar (with the same physicochemical properties) amino acids in the sequence. Methods based on artificial intelligence and machine learning could be very useful if there is enough amount of precise data. The approaches used to assess allergenicity must take into account the known immune mechanisms in the human body and assessment decisions	A more detailed information about the type of the allergens (major or minor), known T cell and IgE epitopes, rote of exposure and origin as well as knowledge of the protein sequence and structure will improve the performance of the in silico models derived on these data. A clear definition for non-allergen and even a precise list of defined non-allergenic protein will help for the assessment of the in silico approaches.	The individual proteins are the most relevant if we want to compare the results or apply in parallel different type of methods: in silico, in vitro, in vivo.	All the factors that affect the allergenicity should be considered in the allergenicity risk assessment.		The most effective methods for allergenicity prediction are both sensitisation and elicitation.	In silico assays should always be used. They are not time and money consuming. In vitro assays should be used to confirm a positive result from in silico assay.	The best validation of in silico and in vitro models is with ex-vivo assays.		When weight of evidence approach is used every case should be assessed individually and with respect of the results of the assays.
18			Regular matching with the Ministry of Health data of the countries can be achieved. The public should be made aware.									
19	the data would be very useful because it helps in ensuring that information-rich and reliable is collected for proper policies to be drawn and data-driven decisions can be made easy too. proper risk assessment approaches could be made from the available data and most suitable proteins can be provided to mitigate or reduce allergic reactions.	FASTA is use in nucleotides or amino acids sequence and are represented using single-letter codes. The format also allows for sequence names and comments to precede the sequences I believe Fasta is a better & flexible for DNA sequence searches. It provides small word sizes to accommodate polymerase chain reaction primers having short sequences and I would prefer its continuity.	I use WHO quite often compare to allergen databases because of its accessibility and user friend.	Raw materials express the intact protein, which means both PCR and immunological tests can be used. However, once a raw material has been processed, its proteins denature with the result that they change their natural structure and in most of the cases cannot be detected by immunological tests.	some strategies taken to improve the performance of relevant and also had a huge impact on the application of enzymes in food and feed. Some of which are able to retain stability under temperatures that are very high either a screening test for the assessed feature is performed	I believe these issues are very critical and the only way we can overcome it is through early diagnosis and serious caution during food intake. Strengthen the issue of correct and very representative labeling might help.	Prediction and evaluation of the potential allergenicity is of importance for safety of foods and other environment factors. Although several computational approaches for assessing the potential allergenicity of proteins have been developed, their performance and relative merits and shortcomings have not been compared systematically but I believe sensitivity is the most effective way.				this can only be done when level are adhere to and proper and standard labeling formats are used.
20	Allergenic risk assessment of novel protein is currently performed using in silico methods and in vitro digestibility tests. These methods are giving indications on a putative risk, however it is known that protein digestibility is not always correlated to allergenicity. Analysis of available data human data on sensitisation and elicitation of allergic reactions might be used to better understand these mechanisms and eventually to design better predictive methods for risk assessment.	FASTA algorithm such as 80mers sliding window are useful for getting a first hint on potential cross reactivity, however a positive result with this analysis should be further investigated in relation to protein domains, for example a hit including a signal peptide, that will be cleaved, is probably not relevant, while a hit with a similar protein domain (that will probably have a similar 3D structure) is relevant. The 8mer (100% homology) defined because it is the minimal length of an epitope is not useful because it produces too many false positives, the presence of only one 8mer hit is probably not sufficient to trigger an allergenic reaction.	COMPARE is effective for FASTA searches, the database is well maintained, The process for including allergens is transparent and well defined. Possible improvements could be if the data is available to describe the different domains and heir involvement in allergenicity I guess this is not always possible, and costly in terms of time and ressources.	Individuals proteins seems to be the best material. Extracts will contain other compounds that could produce artifacts.				Allergenicity prediction should not be decided on a single experiment, it should be based on a weight of evidence. If in silico tools are predicting a risk then in vitro assays should be performed. Resistance to pepsin alone is not sufficient to predict allergenicity at the opposite sensitivity to pepsin alone is not a guarantee that the protein is not allergenic.	?		

21	A large risk is cross-reactions to the most common allergens such as peanut, hazelnut and shrimp. Innovative proteins from e.g. other legumes than peanut or insects might provoke allergic cross-reactions which can lead to the same severe symptoms as the original allergy. Data on prevalence to the most common food allergens is thus important. Also, in vitro assays to study IgE-antibody affinity or oral food challenges to new proteins are important.	My knowledge regarding this is too low to answer the question.	My knowledge regarding this is too low to answer the question.	If used in an in vitro assay an extract or individual protein would probably be the best. But a combination would also be good. It is important to adress the uncertainties with the different test materials.	All that can occur to/within the food. Pea proteins are currently used as meat replacements and these might be developed by high pressure. Can this lead to cross-reactivity although peas are tolerated by penaut or soy-allergic patients?	The two cases described but also regarding "new" food proteins e.g. pea protein as meat replacement.	Elicitation is most effective to prevent harm. Sensitisation does not automatically mean that a person will develop symptoms.	-	-	There is a need for better follow up of allergic reactions in patients. The health care seldom has the time though. Co-operation between national food agencys and the health care could be important. This could also help to increase the knowledge regarding unexpected allergic reactions to the most common food allergens such as peanut, hazelnut, milk and egg.	This a risk management action and outside EFSA´s responsibility. But it would be very beneficial to adress when there there is an extremly low risk of an allergic response. It is better to communicate to the risk managers that there is an extremly low risk of an allergic response compared to if it is communicated that it cannot be excluded that an allergic response can occur. Different levels of risk is very important to communicate to the risk managers.
22		I think the current fasta algorithm is good depending on the parameters that are set. In some cases, it produces sequence comparisons that are highly conservative and contains a lot of false-positive information. Finding ways to target important sequences and putting them in the structural context of response elicitation and progression would make fasta a better tool.	The current databases seem to be fit-for-purpose, although there are some discrepancies among the outputs of the various sources based on the same sequence. I think mapping target epitopes for allergens, rather than full-length sequences might be useful to help eliminate false-positive results and focus in on sequences that may have real hazard potential	The putative allergen is the protein of interest, or the sequence within the protein of interest that has a significant match to known allergen(s). things like extracts, processing, matrix effects, etc. all can impact exposure to the protein of interest or the sequence within the protein of interest.	It has been shown that there is not a good correlation between degradation by digestive enzymes and true allergenicity.	I think human sera should be used when needed, and when available, and alternative methods should be used as soon as there are validated alternative methods capable of providing a reasonable prediction of a real IgE response.	The methods currently being used seem to be relatively effective, as there is no evidence of an increase in allergenicity in human populations related to the introduction, cultivation or consumption of any genetically-modified plant in commerce today.	in silico or in vitro assays should be used when they have been validated and shown to be predictive of allergenicity. It is not feasible or reasonable to implement novel technologies or processes into a regulatory safety/risk assessment without understanding the sensitivity and specificity of the assays, the results that predict an adverse outcome, and how regulatory decisions will be promulgated based on the outcome of such evaluations. When such assays exist, they should be implemented in a tiered approach, based on a problem formulation, and using a weight-of-evidence.	It takes time and investment. Two things that seem to be in short supply globally.		
23	Using big data and in silico analytics	No idea	No idea	No idea	No idea	No idea			By creating simple, controllable test and assays for initial validation. Implemenbting a layered approach to validation where hypotheses are tested in a piecewise fashion.		
24	Proteomics can be used to characterize the specific IgE binding site that allows for proteins to bind and elicit their allergenic reaction. Bioinformatics can be used to assess whether these IgE binding sites exist on novel proteins, which, if there exists human sensitization data, can be used in a read-across approach to assess whether the novel protein contains any potential for allergenicity.		The current databases are useful but would be improved if there were structural 3D databases to evaluate the binding site of proteins.	Individual protein is the most relevant test material as whole food matrices or extracts may contain other unintended compounds that may elicit allergenic reactions.	Food processing, digestibility studies, temperature and pH stability tests should be considered in the allergenicity risk assessment as these would provide "true" or "actual" scenarios under the conditions of use of the food. Digestibility studies can be advanced - full gastrointestinal tract scenarios (INFO DIGEST).					
25	This depends on who the subjects are. If just the normal population then its a good start so long as you have a large cohort. You would want ethnic groups, other allergen sensitive individuals etc proportionately covered and suitably randomised. If the in-silico assessment from the WGS/proteomic assessment indicates higher at risk groups from cross-sensitisation etc., then they should be used for more targeted studies. Depends on whether you are trying to prove a negative or a positive in approach.	Not my specific area, so would leave it to experts to respond	They seem useful to EFSA and easy to use. Independent review would be the best answer to this question.	Obviously a tiered approach using a combination. Starting with in-silico assessment	All of them.... Up to the applicant to justify	Case by case. They are part of the overall data package. I am not sure what else you can do to replace them in similar circumstances	Probably both	Good questions, interested to see what the experts say	This depends on whether you are trying to prove a positive or a negative. But I am not an expert so interested to hear others	One suggestion is for first X months or years of a new plant ingredient being introduced it is highlighted on the label or has "contains X" then at least you stand a chance of gathering data once introduced.	Very very good question to which I would have liked an answer for the last 20 years.

26	To extrapolate existing human data to predict sensitization of new proteins is tricky. It depends on the level of similarity of the protein composition of the novel protein in comparison with known allergens and of the quality of the clinical data and level of details about the protein composition that was used to conduct a challenge test. Sera of allergenic people can be used to check for IgE binding to the novel protein, but this only gives an estimation on potential cross-reactivity and not the possibility of completely de novo allergenicity	I do not have experience using this tool but it apparently provides the most predictive results for protein sequence similarity	They are all powerful tools, but aimed at predicting potential cross-reactivity with known allergens. De novo allergenicity is very difficult to predict with these tools, but perhaps with a combination of protein characteristics, like digestion or physico-chemical and biological properties	A combination of all these, as it is important to understand what particular part of the protein in the whole food (raw or processed) my elicit the allergic response. However matrix effects and also processing may greatly affect the protein characteristics and therefore allergenicity.	They are all of value	A tiered approach should be applied depending on the level of accuracy of the prediction. I think in first instance there is a good rational to do a small scale in vivo test to corroborate the in vitro findings. In second instance the hit needs to be checked first making use of human sera samples.	Sensitization is the key step, but without recurrent exposure to the allergen there is no elicitation, besides elicitation is important to asses the potency or severity of the response. So elicitation is key to prevent harm	To assess cross reactivity they should be done if sufficient level of similarity has been reached. In that case it would be a second tier and the last tier would be in vivo challenge test. For de novo allergenicity protein characteristics of the novel protein can help, but not sufficient. This should be combined with in vivo testing to really reaffirm.	not with animal models, but human data so ex-vivo and in vivo	Post market surveillance tools & process, with a good database for case reporting, to keep track of potential development of a new allergenicity	There is a lot of information in the scientific domain on potential threshold levels in the actual allergenic population, on most of the known allergens, with only a very small amount of the population (1% ideally or 5%) eliciting a minor response. This is a very good basis and there is only more data added to the existing information which only further strengthens this approach. Level of quality of the data is very important, the amount of people included in the population and accuracy of the data (level of health response (IgE confirmed), type of proteins tested etc.)
27	clinical data is needed to determine if proteins are allergenic or not and if allergens are weak,strong or intermediate allergenic. Tests should be clinical relevant and predict for sensitisation and/or elicitation. You could use prevelance of sensitisation or of elicitation as measure for allergenicity	thresholds of homology/identity should be reconsidered escpecially for novel foods. The test was never developed for novel foods. Now very conservative proteins are poping up requiering IgE binding tests for very rare allergens	No, they contain to many fragments and rare allergens, which lead to false positive hits. The evidence is to small for adding some proteins to the list. Solely IgE binding in in 5 sera is not enough	the product that is going to be eaten, including matrix and processing. But then protein extraction can be difficult. So in that case the next best option	only methods that predict for allergenicity (and allergenicity should be defined first). None of the mentioned tests are predictive.	It is very difficult to find sera with IgE against a certain allergen especially when it is a minor allergen. Also tests with this rare allergen (diagnostic tests) are not available. Use sera from the allergenic source (more for minor than for major) regardeless of IgE. Start with a good bioinformatic tool and database to minimize the need of sera.	most practical is sensitisation (especially for individual proteins)	A method should be predictive for the harm you would like to prevent. If one method cannot do this than a combination of methods will give you more information. Methods should be validated. The methods mentioned are not predictive e.g., 35% similarity with an allergen, resistance of a protein to pepsin over X minutes). We need to find better methods, but this depends on the harm we would like to prevent. That determines the ranking of the allergens from weak to strong and thus the reference set, which can be used for validation	We first need to decide where these methods, need to predict for so, which harm do we want to prevent. That determines the ranking of the allergens from weak to strong and thus the reference set, which can be used for validation	discuss with clinicians which data should be gathered to determine if the adverse reactions are related to food allergy. Most of the time the wrong information is collected. Even better to collect sera.	use clinical data and product data
28	The available human data on sensitisation to known allergens means that we know the clinical manifestation of allergic reaction and we can harvest the sera from the sensitised persons, that can be used for assessing the presence of allergen-specific IgE as well as we can perform molecular analysis for developing innovative proteins design for allergy diagnosis but also for new immunotherapies. Correlating the clinical data from sensitised persons with respect to the severity of the allergic reaction, with the in vitro test (eg. basophil activation test) we can predict which allergenic molecules are associated with the risk of developing severe allergic	FASTA is a DNA and protein sequence alignment software package hosted by European Bioinformatics institute, which was designed to use at the beginning local sequence alignment and then extends to global alignment. FASTA works better for similarity searching in less similar sequences, and is indicated especially for nucleotide searches. Another tool is BLAST, which is an algorithm for comparing primary biological sequence information (nucleotide or amino acid), works better for similarity searching in closely matched sequences, and is indicated especially for protein searches. BLAST searches for similarity between a query	The current allergen databases (e.g., Allergenonline, Allergome, Allermatch, Compare, WHO/IUIS) are effective for allergenicity assessment, but they can be improved to better support the risk assessment by in order to find the T- and B-epitopes responsible for sensitisation and for developing severe allergic reactions to different allergens. Moreover, the allergen sources are increasing, and new food is one of the most challenging source of new allergens.	the most relevant test materials to predict allergenicity is a combination of individual proteins, extracts (raw or processed), whole food matrix, in correlation with the clinical data.	The allergenicity risk assessment in this moment is based on molecular stability parameters (e.g., effect of processing, enzyme degradation studies, temperature, pH). In the future we should assess also other physicochemical properties (e.g., glycation, aggregation, pressure stability).	Human sera should always be used to assess the allergenicity of a certain component because it gives the relevance of the molecule for characterizing it as minor vs major allergen. This way is finally important even for the potential allergenic molecules identified following bioinformatic analysis.	The most effective methods to predict allergenicity are both, sensitisation and elicitation, and this is already known for many allergenic foods.	In silico and in vitro assays should be used when are available (including trained personnel) to better support allergenicity prediction. Their results can then be translated into clinical studies.	The best to validate in silico and in vitro methods with animal models, ex-vivo models and others is by going into clinical studies.	Post-market monitoring tools should be developed in cooperation between researchers and clinicians involved in treating allergic people (better with the academic societies).	Acceptable levels and/or thresholds of protein that will not raise safety concerns about cross-reactive or de novo allergic responses is also a subject of collaborative research between bioinformatics, food producers, clinicians and academic societies, and accredited safety food entities.
29	Available allergen protein sequences of soy, fish, nuts etc are used by several regulators in allergenicity assessments. Further, these sequences are very useful for studying the in-silico allergenicity of major protein fractions of a novel protein.	I haven't used FASTA yet.	Yes. Current database can be updated with more sequences to improve the sensitivity of the in-silico analysis.	Both individual proteins and whole food matrix, and also probable formulations where the novel protein will be used - e.g if the protein is intended to be used in infant formulas etc. Studying the allergenicity in all these matrices will be crucial.	Molecular stability parameters for allergenicity: Effect of pepsin digestibility Effect of pH and temperature Denaturation effects Physicochemical Solubility Conductivity	As said in the question, the human serum studies must be done if a hit is identified in bio informatics studies. Further, in-house enzyme digestibility studies also help identify potential allergenicity with the novel proteins. In these cases, human serum analysis must be done with statistically significant samples. Effect of other allergens Vs novel allergen can be documented. Also, data on adverse reactions must be documented	Bio informatics Animal studies followed by human serum analysis				
30	It can be used as a predictive tool when homologies with key epitopes are detected	FASTA provides a useful tool to check for potential homologies with known allergens as a first sign of potential issues. It is a tool that developers use to check if the intended proteins may pose allergenicity issues.	Yes, they are. Databases that are curated to contain proven allergens are most useful, to avoid an excess of false positives	Purified proteins, to avoid confounding effects due to matrix	This should be done in a tiered manner and applying the weight of evidence approach. First checking the protein's history of safe use, then if there are homologies with known allergens and if the protein degrades quickly in simulated gastric fluid. If concerns arise, then glycosilation and processing fate can help in predicting potential for elicitation of an allergenic response.	Human sera is precious, it should only be used in instances where there is a strong concern that cross-reactivity could occur.	Potential cross-reactivity.	Following the problem formulation and weight of evidence approach, these should be conducted only when triggered by the results of homology searches and the protein is not readily degraded in SGF.	Through a lot of research and ring testing.	It is unlikely that a protein suspected of causing allergenicity would be developed and even more unlikely that it would be marketed	The current thresholds are already very conservative. Additional targeted research using known allergens may help in gaining a better understanding.

31	Realizing that humans can not be used as 'guinea pigs', and that there is a societal wish to reduce animal testing, we ill have to rely more on (the development of) in vitro predictive systems. Existing human data can, and need to, be used for validation of such in vitro tests; particularly to develop tests for sensitisation.	It gives directions to think towards; but their relevance is limited and needs biological validation	They are the best available, but not perfect; and need continuous improvement, preferably in co-ordinated efforts to develop also self-learning AI systems and the like. Biological validation (IgE-binding-based tests) remains essential	A combination of all of these: - Individual proteins give the çleanest' view, but (sometimes) not the real answer - There are synergistic effects, at the least sometime e.g. in peanut, in multiple-protein preparations - Matrix components, e.g. lipids, may exert adjuvant effects	The ones that, based on e.g. literature, have shown to be of influence; and that comprises anyway (proteolytic) degradation, processing-related parameters such as aggregation glycation, hydrophobicity changes, amyloid formation	A sufficient number of human sera ('sufficient' means allowing coverage of as many epitopes as possible; isn't 15 or so rule of the thumb?) should be used. I do not know of alternatives: (monoclonal) IgG is irrelevant, in my view, there is too little monoclonal IgE available at the moment. Statisticians need to be involved also in experimental set-up.	We should talk about what level of risk is acceptable; zero-risk is not realistic, so think of 'cut-off' risk values. I think that, for elicitation, there is at the least some level of methodology, which is the best available and workable. For sensitisation, an effort needs to be invested in development of methodology.	The 35% similarity is already an in silico-effort; I think further testing should be done in case of 'alerts' from other predictive tools, and protocols or SOPs should be developed.	Assess a variety of markers, and use tools of multi-variate analysis to relate these		Such levels vary for different countries/populations; so testing-testing-testing, as for covid-19, is necessary.
32			Allergenonline - helpful tool for identification of potential, putative allergens. However, the risk assessment of identified allergens has to be performed based on available literature.								
33	Novel proteins are continuously coming into the food chain via mutations occurring in crops and animal genomes, introduction of micro-organisms and insects products into our food chain and food additives/GMOs/biopesticides. I am not aware that important issues have arrived by the inclusion of these new proteins in our food which would suggest that the current procedures are sufficient. It would be important to align the risk assessment approaches between the different sectors so that there is not a more stringent assessment in one sector compared to the other as this would result in unnecessary use of resources (incl. sera)	A full FASTA is useful as a TIER1 evaluation (together with the information on the source organism and the protein family) and this is the main tool used in most sectors and most countries. Although the 2010 EFSA GMO allergen guidance states that "the added value of initial amino acid sequence segmentation into overlapping 80-mers prior to alignment is questionable" (Annex 3, page 92) EFSA is still requiring this. Either EFSA should provide added value of this practice or align with other sectors and countries and accept the outcome of full FASTA. TIER2, in case of a hit from TIER1 should then be more	These databases are sufficient for a TIER1 screening	Experimental testing using sera or other natural resources should only be used when there has been a TIER1 and TIER2 bioinformatic analysis that considers the epitopes or protein domains known to be able to induce an allergenic reaction	These stability parameters are only relevant when a hazard has been identified as these parameters relate to potential exposure. When no hazard has been identified they have only limited additional value.	When the protein is from an allergenic source this is not sufficient as a trigger as the protein family and the bioinformatic analysis could show that the selected protein is not the cause of the allergen property. As such this trigger is not sufficient to suggest a hazard as not all proteins from an allergenic source are allergens. TIER1 bioinformatic analysis (FASTA) should be complemented with TIER2 analysis that for example looks at the which part of the NEP and the allergen is aligned and if this part of the protein has as high level hits to non-allergens and potentially use more sophisticated models.	Looking at the protein family	The information on the protein family should get a much higher weight in the risk assessment (for example if this protein family is not know to harbour allergens)	Only validated methods should be used and that it is clear how to integrate the information in the risk assessment and this process should be used across all EFSA units that risk assess proteins (or organisms)	As GM foods are almost not available on the EU market these tools are not useful	Has an applicant ever request to put a protein with a clear hazard on the market, if not it would be better to put efforts to develop tools to assess false positive hits (high level known to occur)
34		FASTA is the most basic homology analysis algorithm. If an alternative approach is used, it should initially be evaluated against the results of FASTA searches. If, in the future, an alternative approach is established that is clearly more useful than FASTA, then the use of FASTA can be discontinued.	Allergen databases are effective in that they consolidate information on amino acid sequences, epitopes, three-dimensional structures, protein functions, etc., for known allergens, and these information can be compared and searched at once. However, the allergen database needs to be equipped with more accurate allergenicity prediction methods other than the FAO/WHO methods.		The molecular stability parameters currently used for evaluation (effects of processing, enzyme degradation studies, temperature, pH, etc.) should be considered.	The interpretation of IgE-binding results is difficult because even in patients with allergies to the same food, the allergens to which they react may differ and the severity of the allergic reaction may vary. It is desirable to standardize the method of interpretation.	Even if a false positive occurs, it is important to avoid false negatives.	In vitro assays other than IgE binding assessment and in silico assays should always be performed.		It is necessary to establish a highly accurate and comprehensive information collection system.	
35	We have to differentiate between research findings using well-characterized study populations and studies that do not have well-characterized patients, especially the serological outcomes that are not from patients whose allergies have been confirmed by trained allergists. (additional comments: With the electronic system of taking patient history, some of the patients have food intolerances that are generally characterized as "allergies" and some the the sera may inadvertently end up in research findings that are not as useful. With this differentiation, we can have more meaningful findings to improve the	Explore in silico methods of predicting which algorithms would explain the true predictions (and exceptions) of the pepsin digestion assay used in predicting potential allergenicity of novel proteins.	The current databases are useful. Improvements would be to define the limitations of each database and/or the applicability of each database to which types of assessments.	Individual proteins give the most precise information in terms of allergenicity. The effects of extracts and/or whole food matrices or a combination can be explored as additional research interests.	The current molecular stability parameters being used give relevant information about novel proteins.	Bioinformatic hits are not always perfectly predictive of actual risk. They are an indication of the possibility of risk. Serological tests may be warranted based on expert assessment of the potential risk being evaluated.	The most effective methods to predict allergenicity would be methods that evaluate elicitation. There is clear evidence to show that sensitisation may not always be followed by elicitation. In addition, the elicitation parameters are clear cut and less ambiguously measurable. More research efforts can be put in sensitisation with the longer term goal of developing methods of assessment that can be validated and shown to be predictive within specified limits.	Based on expert assessment of the overall risk that could possibly be encountered with regards to the protein in question.	Validation can be based on already existing food proteins that have been shown to be safe by virtue of being consumed over decades, centuries or millenia. We have data on their safety, so if some potential methods come out to say these older food proteins are not safe, then we can use this information to define the specific situations for which these methods or their outcomes may not be predicting accurately.	Current tools are effective enough.	Based on current consumption data.
36	Based on physicochemical and biological properties of a new protein, the available data on know allergens can help to predict the effect of the new proteins on allergic reactions.	Sequence alignment is required to determine potential allergenicity of a protein. Comparison with known allergens is the base info needed.	Only experienced with AllergenOnline. Only clinically relevant proteins/sequences are withheld, and "scrap proteins" are scrutinized by a panel of clinical experts. Other databases that screen all proteins in the world also come up with scrap proteins, or proteins that are mentioned in allergenicity studies but that are not allergens on their own. Those databases (e.g. NCBI or Blast) should be avoided.	Immunoblots require a minimum concentration of proteins. By using different methods of extraction and purification (e.g. ultrafiltration), the protein levels can be increased to a level that is needed for the immunoblots. Testing single ingredients may result in overlooking clinically relevant proteins.	Processing of the product that contains the new protein can help to deactivate the protein and its allergenic propterties. E.g. heat treatment, pH or high pressure alters the protein structure. The presence of enzymes in the final product can also affect the allergenic properties. Any treatment can have a beneficial/worse affect on the final allergenic properties of the food.	Homology screening results frequently in a large amount of homologous proteins. The availability of enough target serum is limited to perform the required number of immunoblots. A prediction model should be developed to check only the most relevant sequences in the immunoblots so that the number of tests can be limited. The homology window screening should be reconsidered (80 amino acid long sequence, min. 35% homology) as it results in a large amount of homologous proteins.	Elicitation is most effective. A potential allergenicity can be overlooked when tested in people that have no allergies, whereas it will be quickly picked up in people with known allergies.	Homology screening usually results in a large amount of sequences. The number of in vitro tests that should be performed is too large, addidtional/adapted thresholds (via in silico analyses) should be included to limit the amount of in vitro tests. Pepsin degradation assays are not reliable and are not indicative for allergenicity.	Are animals representative for humans with allergic reactions?	When a product is marketed, either consumers can be contacted via consumer surveys to check for potential allergic reactions after consuming the novel food. A sentence could be added on the pack that informs the consumer to contact the company/authority to flag potential allergic reactions towards the novel protein.	Establish thresholds both on the total amount of proteins in the food, and the amount of the different individual proteins. If the total amount of protein is limited and there are many different types of proteins present, the chance is low that a allergenic protein is above a level at which it could provoke the allergic reaction. Data to be generated. Daily intake of the food is to be considered as well in this approach.

37	Bot data on occurrence of allergenicity to certain proteins and their associated thresholds may allow to estimate the risk of cross-reaction to these innovative proteins (which were shown to be similar to the proteins for which data is available after BLAST analysis)			All of these are necesary to allow a good evaluation * Individual proteins allow sequence determination and make identification of PTM's a bit more easy * Extracts of raw and processed proteins allow to evaluate the effect of processing * Whole food is necessary as matrix compounds might have an effect on e.g. digestibility, but also here matrix compounds have an impact on the proteins during processing (e.g. Maillard reaction)	Enzym degradation studies and effect of processing are important factors to be studied. Results from literature seem to indicate that pressure might have an effect on allergenicity. So this might also be an important factor to be considered. Pressure stability might also be considered as part of the impact op processing.						
38	By comparative analysis and assessments.	I have not tested FASTA for allergenicity assessments, however I know that FASTA is a powerful tool to search for protein domains. I assume that other tools for protein domain searches could be used, or could be used after algorithm modifications.	I can not provide the answer. I do not have the needed expertise.	I guess whole food matrix because of possible synergistic/antagonistic effects.	Protease digestability.	Can not answer. Not my field of study.	I guess both.	In silico always, in vitro when certain other predictive tools have exceeded a threshold.	In silico as a good indicator, in vitro as further proof of concept.	Post market monitoring should be designed in such a fashion that it allows reliable detection. If the alergen or potential source of the alergen is detected, their presence should be clearly disclosed. It could be very hard to determine the minimum allergenicity dose for different individuals.	Very hard :)
39	Data on the main sites and routes of both sensitization and elicitation are essential in order to understand the risk and potential mitigation of risk without resorting to simple avoidance		Yes, these databases are helpful.	A combination of test materials is needed to understand the risks posed by relevant scenarios	The effect of processing including thermal are important but only in the context of realistic processing scenarios including interactions with other components. This is particularly relevant to glycation and other similar post translational modifications. The stability to protease activity is particularly relevant.		Both sensitisation and elicitation are important in predicting allergenicity. In the context of risk assessment, exposure is a key parameter.				
40	The available human data on sensitisation and elicitation of allergic reactions to known allergens can already be used in screening for risks due to potential cross-reactivities of innovative proteins with known allergens. When looking at the de novo sensitisation potential of innovative proteins, the available data could help inform more advanced in silico approaches for de novo allergenicity assessment.	It is helpful for potential cross-reactivities but not for de novo sensitisation. More in silico approaches around prediction of protein structure and binding patterns could enable more information regarding the assessment of de novo sensitisation.	They are helpful for potential cross-reactivities but not for de novo sensitisation. More out-of-the-box approaches looking at known allergens in relation to other proteins, such as those published by Westerhout et al 2019 (https://doi.org/10.1016/j.yrtph.2019.104422) could help in combination with other in silico approaches to enable more information regarding the assessment of de novo sensitisation.	It really depends on the on the situation, so I would have to say a combination of these. Forms relevant to production of the protein/food (regarding potential workplace sensitisations) as well as forms relevant to how the protein/food is sold to consumers (raw/process, whole food matrix) could be considered. However, if the product is an ingredient to be used in multiple final food products of different natures then it will likely be impossible to assess all final food matrixes and a selection should be made.			Both, but clearly there will be no elicitation without sensitisation.	If we are talking about single proteins, then only when other predictive tools have exceeded a thresholds. If we are talking about whole foods being newly introduced into the diet, then more methods may be needed as the assessment would not only be about the results of a single protein and as such the two examples of predictive tools do not easily apply to whole foods.	This is a difficult question as the purpose for validation is left open. Is the purpose to rank a protein/food along a sliding scale of foods seemingly known as strong/weak/not allergenic? Or to predict if anyone will become sensitised? What percentage of sensitised individuals will experience allergic symptoms upon consumption of the protein/food during exposures from its intended usage levels? Or what is the minimal eliciting dose in a sensitised individual? In all situations an agreed upon benchmark set of proteins and whole food samples available for testing purposes would be a start.	How is not my specialty, but it is clear the post-market monitoring tools should be available.	Initial research regarding levels of individual exposure and a proof-of-concept data framework for establishing a "Threshold of Allergenic Concern" have been completed. In short, there seems to be a potential level of exposure, below which no proteins have been identified to be an allergenic concern. While this concept is not fully completed for all identified allergens, it is a promising start that could greatly simplify the risk assessment process and direct resources in a more efficient manner. Hopefully Dr. Clare Mills would be able to share more information on the progress of this research as it was presented by one of her students at an
41	Serum of patients known to be allergic to several foods could be tested against potential new allergens and if there is a reaction, this information can help understanding that patients allergic to X food, may also be allergic to the new protein.	My understanding is that allergenicity does not depend only on the amino acid sequence of proteins and the structure of the protein (not identified with FASTA) is also very important. Hence, the information obtained from the comparison of the WGS against allergen databases seems like a first step in the allergenicity assessment. One can conclude from this assessment that there may be a potential of allergenicity but not that a protein is allergenic.	These databases are definitely important to keep track of allergenic and, as in question 2, may give a first idea of whether a protein may be allergenic due to similarity to known allergens. However, new (unknown) allergens are not included in these databases and many potential allergens may be missed due to the impossibility of comparing them with known sequences. To better support the risk assessment, the databases could be harmonised, so there is only one database.	I am not an expert in this field, but I believe protein extracts. It may be useful to	The effect of processing may modify the structure of a protein and change the allergenic potential. It's been reported in the literature that physicochemical properties such as glycation and aggregation can increase allergenicity and these should also be considered. However, I am currently unaware of the techniques that can be used to study these properties.	I actually see that serum analysis can give more information on allergenicity than the bioinformatic analysis proposed in EFSA guidance on GMO allergenicity. However, in order to obtain relevant results, it would be ideal to test individual proteins of the sample. Hence, an initial protein characterisation and isolation would be necessary. But I see here again the same issue as with the allergen databases. If novel proteins are present in the sample (and not present in protein/peptide databases), these will not be identified in the protein characterisation step.	I am not an expert in this field, but perhaps serum analyses would be very effective. Both testing a protein extract of a novel product or individual proteins would help understanding whether the product is allergenic.	-	-	Post-marketing monitoring is key in the allergenicity of foods with novel proteins. The RASFF system can be used to report adverse reactions to novel foods and companies marketing foods with unknowns allergenic potential should report to the authorities any complaints they receive on the products regarding allergenic reactions.	My understanding is that an allergen can provoke an allergenic reaction in a sensitive person even at very low levels. Hence, I don't see a proper threshold of protein that would not raise safety concerns. Labelling will be key here and clear information should be given to potentially allergic consumers.
42	Mapping of known allergenic protein domains can help in-silico analyses of innovative proteins	FASTA algorithm shows on only sequence similarities, however computer annotated/predicted 3D-protein structures can also illustrate how and if complex folded proteins resemble known allergens.	I have not used them so I cannot answer.	In my opition combination of all these.	All of the above, and also interaction with other chemical compounds (herbicides, pesticides, food and pharma additives etc.).	I am not an expert in human immunology, I cannot say.	No opinion	In my opinion in silico assays should always be carried out.	I have mu doubts with animal models, as animal physiology is not comparable to human physiology and immunology. Ex-vivo human tissue models (cell cultures) also lack complexity compared to individuals/test subjects. Perhaps one could test animals and then look for similar responses in ex-vivo cultues (inflammation, cell markers etc.)?	Difficult, as nutrition is complex - finding out the allergenic component might be very difficult - pre-market allergenicity tests are critical.	No answer.

43	Those information is very helpful since they are the foundation of allergenicity assessment. However, those information is very hard to collect and select for use.	The current FASTA algorithm is easy to follow.	There are so many database available online. However, it is very hard for user to tell the quality and the reliability. The EFSA scientific panel can evaluate and select several of them to form a database for applicant to use. It can avoid the complexity of analyse results presented by different applicant. A reasonable E value cutoff should also be set to uniform the scale of the candidate for further analysis.	For a new protein, it should be the protein per se. For an old protein that has been consumed by human, it should be based on the HOSU.	I don't think they help. Normally, we consider the enzyme digradation ability more relevant, however, there is no absolute correction between high resistance and allergenicity.	Scientist has done many work to identify almost all the allergens in the allergenic foods. So only the ones that share high identical (35% for an 80 aa window or full length analysis) to those allergens should conduct such human sera test. For bioinformatic hits, only those with 50% identity for an 80 aa window or full length analysis with low E value (<10E-5) and with positive results of 3D model analysis should conduct such kind of sera test. All in all, the human sera test should be conducted as less as possible only with confirmed evidence.	No idea.	35% similarity with an allergen. But the criteria should be set higher such like longer length, at least 50% homology and E<10E-5.	No idea.We should consult with medical science expert.	Access to medical alarm system for allergenicity.	35% is obviously unreasonable for allergenicity assessment. High similarity and E value may be combined for predict the allergenicity potential.
44	Elucidation of the mechanisms underlying sensitization and elicitation (and the features of proteins that have a role in these reactions) may also aid in the improvement of the prediction of the capacity of novel proteins to cause these reactions. Currently, the risk assessment is focused on potential cross-reactivity plus some generic, weak indicators of possible sensitization as de-novo allergens (e.g. resistance to digestion, overall structural similarity to known allergens). Yet all is based on a weight-of-evidence as there is no sole conclusive indicator. Hence the current assessment would merit from predictive tools that	FASTA is straightforward, universal, and easily applicable to any sequence. Moreover, is has been the method of choice in the internationally harmonized guidelines of Codex alimentarius. Yet FASTA (and other methods such as BLAST) have not been developed for the purpose of allergenicity assessment per se. For example, the scoring matrices (in case of substitutions) are based on evolutionary relationships which may not necessarily be relevant to allergenicity. In addition, it focuses on linear sequences whereas some of the known epitopes are spatial, particularly also those involved in	Many of these databases (e.g. Allergenonline, Compare, Allermatch) can be used to perform allergenicity predictions in line with Codex Alimentarius guidelines one way or another, with each website providing its own "niche" benefit, such as expert review. Some are also mutually dependent, e.g. Allermatch using sequences from WHO/IUIS, Uniprot's allergen list, and Compare as inputs for its own database of known allergenic proteins (being processed by Allermatch, i.e. with propeptides removed in line with Codex recommendations). Guidelines from EFSA for database establishment (used as input for homology	Obviously, it would be desirable to know the matrix impacts besides the behaviour of the individual proteins in isolation, yet it is difficult to see how such tests can be standardized.	These parameters should be linked to parameters relevant to allergenicity, e.g. loss of antibody binding upon degradation, denaturation etc. (or, vice versa, formation of de-novo epitopes during processing).	As for the bioinformatics approaches, one could define supplementary rules to define whether cross-reactivity may occur or not before proceeding to sera testing. For example, multiple epitopes should be present on a protein (or aggregate) before it can elicit a mast-cell reaction after binding IgE, hence a single short stretch of homology is irrelevant or could actually de-sensitize a patient. Another criterion is when a gene source is considered allergenic or not. Does this pertain to only the "big eight" (or the EU labeling list) or is this any source for which there is anecdotal evidence of allergic reactions? In the first case, sufficient sera may be present	Currently, much relies on bioinformatics given the limited predictive power of protease resistance and gene source.	As stated above, bioinformatics is key. Resistance to pepsin by itself does not provide sufficient clues for further testing (yet its absence could provide additional reassurance in the weight-of-evidence approach). If the outcomes of bioinformatics indicate possible allergenicity (e.g. sliding window, FASTA), then one should proceed with supplementary bioinformatics tools and/or in-vitro tests based on the outcome (e.g. cross-reactive epitopes identified)	I would not champion the performance of additional animal and human testing but base the validation on what is already known for particular allergenic proteins that have been well-investigated.	This should particularly be focused on de-novo sensitization (e.g. the example of kiwi provided in a previous EFSA GMO Panel working group report more than ten years ago), an item for which there are currently no predictive tools.	This would be in line with the threshold-of-toxicological-concern (TTOC) approach already established for other types of molecules. Without going into detail, it is important to build in safety margins to account for inter-individual variability.
45	There should be discussion about the clinical, biochemical and testing data used to identify a protein as an allergen. There should be information about the amount of the protein in the food sources, or if from environmental exposure, from the source (pollen, fungus). A major challenge is that simple IgE binding is not proof of allergy, rather biological data is needed such as food challenge, basophil/mast cell activation, with a connection to abundance of the protein in the material. Often purified proteins are tested, leaving out the exposure component. And that is needed to construct a better database of proteins to compare	An overall FASTA comparison is very helpful, but the identity matches should also be tied to other sources of proteins for exposure. How similar (identical) is tropomyosin from a variety of food sources, and from human endogenous protein? What is the sequence identity of Lipid Transfer Protein to LTPs of other commonly encountered food and environmental sources, and what is the clinical history of allergy from the various sources. Allergy to any one source, or protein is a relatively rare event. So it is hard to get even modestly accurate information. Many allergens have been identified that are RARE in occurrence of humans having IgE	The AllergenOnline is pretty accurate as far as selection of proteins that have at least laboratory IgE binding to proteins, and additional information regarding biological activity for many of the major allergens. It uses peer reviewed publications for evidence. There are also a number of proteins that have little data, and/or few subjects that are positive. We could talk about evidence for whole protein sequences... and should talk about that. We also have Full length FASTA, sliding 80 AA window that can calculate % ID for matches less than 80AA and compensate (adjust to equate to 80). It also allows one to select the % identity match (35%, 40%,	Both extracts an pure proteins are helpful, but need to be specified. What is the source? Was it denatured or not, are there full length proteins, and what is the concentration. The source of sera is EXTREMELY important, with clinical history as accurate as possible, clinical reactions and exposure data. Whole food matrix is not useful unless the question is the impact of the matrix, or impact of food processing.	To some extent, but really typically the natural, native conformation is the best starting point. The other questions relate to possible impacts of food processing, and that differs based on food types. For commodities, or crops/animals etc. the proteins are often used in different processed fractions. So I see little benefit in looking at processing unless it is a food source that is ALWAYS boiled or treated by a process that can change protein characteristics, like fermentation. The risk assessment should be based on the native material.	IF there is a clear indication of allergy associated with the source, or the test protein with relatively high identity match to a clear allergen, then serum donors are needed with the right exposure and clinical characteristics demonstrating allergy or at least suspected allergy, and demonstrating IgE binding to the "clearly allergenic" material. Then testing needs to be done with control serum also, and methods that show IgE binding to the positive control, and whether there is (or not) binding to the protein or food source of interest. A thorough test would include IgE binding to native and reduced forms of the protein and source. So an	You cannot effectively predict sensitization. It is too complicated. Elicitation is the primary concern. Is someone going to react or not. And their previous clinical and environmental history is important. What are their symptoms? What are the sources of allergen that cause symptoms. How will they be exposed to the protein(s). By the way, sensitization is not harm. Elicitation is harm. Most people have some IgE to some dietary proteins and environmental proteins. But relatively few have allergic reactions. Many people are falsely diagnosed with allergy simply based on IgE binding. And what is the	In silico (FASTA) can be done on all proteins, cheaply and simply, but we need better criteria and maybe better curation of the databases. IgE binding should ONLY be done if the source of the gene/protein is a common allergen (big 8 or big 14, with selected tree nuts). Resistance to pepsin does not indicate possible IgE binding or allergy. Exposure to the source is important. Testing IgE binding for pepsin resistant proteins does NOT make sense, unless the protein is from an allergenic source. Source and Bioinformatics (usually greater identity than 35% over 80) should be the PRIMARY evaluation.	Validation should be done based on human clinical history. What is an allergen, does the method predict allergenicity? Animal models provide a research tool that can help understand differences or mechanisms and possibly alteration of allergy. Do they accurately predict sensitization? Maybe a few do, but how many people are exposed to peanut and how many are allergic? How about rice? Can an animal model accurately predict that? Maybe an animal model can help understand clinical reactivity, if the right dose/exposure and antibodies are made. Maybe a transgenic mouse or rat with human FcERI can help demonstrate cross-	Post market monitoring (PMM) is highly unlikely to be useful. Especially unless the food is commonly used and identifiable. Post market monitoring did NOT work for the artificial sweetner, Aspartame. In designed trials. Post market monitoring has not worked with Quorn, It usually provides false positive responses. Would post market monitoring have helped with Kiwi fruit introduction? Maybe, but at the end of the day, kiwi is generally quite recognizable and risk management takes care of identity and of warning allergic consumers...if they are properly diagnosed. How can PMM help us understand which	Threshold are individualized across consumers and allergens. Some severely impacted peanut allergic consumers can have an OBJECTIVE symptom (usually hives or asthma, breathing trouble) at a few milligrams of peanut protein. Others with severe symptoms may require gram quantities. Quite often the low threshold doses reported for something like peanut by studies published by FARRP/Hourihane etc. only reported minor symptoms. So the threshold are not based on severe reactions, though with people having severe symptoms upon substantial (individual) exposure. Other potent allergenic foods show "similar" Thresholds.
46	There appears to be a paucity of clinical sensitization and elicitation data on single proteins, so it may be premature to speculate on how such information might be used to inform the allergenicity risk assessment of newly expressed proteins in GM crops.	FASTA is a very useful tool for identifying alignments that are likely to share physiochemical properties that might lead to similar binding by antibodies including IgE, but the current implementation of the method using sliding-window searches and percent identity has been shown to be far inferior to using percent similarity and E-values as designed into the FASTA program. Existing knowledge overwhelmingly supports changes to regulations to align with this knowledge. Of critical importance to developing additional bioinformatic tools, is identifying those allergens with the greatest evidence supporting clinical relevance. Current	The Allergenonline and COMPARE databases are good for conservatively assessing the hazard of newly expressed proteins in GM crops as this was the primary purpose for their development. However, more granularity in the description of the strength on evidence that each sequence in the database (full-length or partial) causes allergy would facilitate development and validation of bioinformatic tools by allowing researchers to restrict the sequences used in such investigations to those with stronger evidence of biological activity.	The appropriate test material may dependent on the particular assay and its purpose. It is not possible to select a test material without first selecting the test system and objective of the assay. The best test material for a specific assay to inform the allergenicity risk assessment should be determined empirically based on is superiority in predicting allergenicity. This should be part of the assay validation process used to determine if the assay system is fit for purpose.	These properties are mostly useful in predicting exposure. With the exception that increased oral exposure to allergens at a young age reduces sensitization later in life, it is not currently understood what exposure scenarios (timing, route of exposure, and concentration) favor sensitization vs. tolerization. In addition, none of the listed properties has been shown to correlate with the allergenic status of proteins. With this understanding, none of these parameters is currently reliable in assessing the allergenic risk of newly expressed proteins in GM crops because no weight can be assigned to them in the weight-of-evidence	Serum screening is currently a viable method to assess the significance of 1) bioinformatic relatedness between novel food proteins and known allergens or 2) proteins sourced from allergenic organisms. If very few serum samples are available, this implies that allergy to the source organism or a bioinformatically similar protein is quite rare making the overall risk very small as well. Appropriate interpretation of assay results requires adherence to proper experimental technique, good experimental design, and interpretation based on magnitude of response and its statistical significance, similar to any other experiment.	Currently, 1) bioinformatic relatedness to known allergens, 2) the allergenic status of the source organism for the protein, 3) any history of previous exposure to the protein, and 4) the concentration of the protein in food are the components of the weight of evidence that currently inform the allergenic risk assessment of newly expressed proteins in GM crops for both elicitation and sensitization. Identification of new allergens with sequences unrelated to sequences currently in the AllergenOnline or COMARE databases are uncommon, so bioinformatic investigation is a powerful tool for predicting allergenic	This depends entirely on the what in silico or in vitro assays are being considered and how predicative they are for allergenicity. It is not possible to provide a generic answer. The key is validating the sensitivity and selectivity/specificity of any component of the weight-of-evidence prior to its use in risk assessment.	Good predictive animal and ex-vivo models for allergenicity are not currently available so this question is currently difficult to answer. Method validation must currently be conducted for 1) sensitivity using known allergens, and 2) specificity/selectivity using proteins with low allergenic risk (where exposure is known but allergenicity is not reported).	Since traditional breeding typically produces more different and less characterized protein profiles and yet has an extremely long history of safety, post-marketing tools applied to GM crops is risk disproportionate unless a hazard is indicated. Only GM crops with negligible allergenic risk are commercialized so post-market monitoring seems scientifically unjustified at this time based on the proportionality of risk.	There is scientific literature available on this, along with descriptions of the difficulties in determining a generic threshold for elicitation or sensitization that could be applied to a novel protein with low risk based on bioinformatic results. It is known that less exposure to allergens in sensitized individuals reduces the risk of eliciting allergenic symptoms, but low levels of exposure can also reduce symptoms when later exposed to higher doses in sensitized individuals. Determining a threshold of concern would be useful in the risk assessment of newly expressed proteins in GM crops but would require extensive research.
47	The use of databases of known allergens represents a valuable tool to predict the allergenic reactions.	Currently FASTA algorithm represent the reference methodology to compare protein sequences. It is possible to use software to predict the structure-function of novel proteins based upon available information on known allergenic proteins and peptides	The current allergen database represent a satisfactory tool to assess allergenicity. The updating of the existing databases is essential to appropriately support the risk assessment.	If available the test material to predict allergenicity is represented by whole food.	The main paramenter are: effect of processing, enzyme degradation studies.	I have no adequate know how to reply to this question	I have not appropriate know how to reply to this question	I have not appropriate know how to reply to this question	I have not appropriate know how to reply to this question	I have not appropriate know how to reply to this question	I have not appropriate know how to reply to this question

48	Allergens (unlike toxins) have differential sensitization based on the genetics and environment of the patient. Any available human data might be applicable maximally to that particular population and the results cannot be extrapolated globally. Allergen avoidance is the best strategy to mitigate allergen risk. E.g. A person allergic to eggs should avoid eggs rather than being worried about the individual allergen levels.	Although the current FASTA algorithm is sufficient enough, the 80mer windows (sliding window search) creates artificial groupings unrelated to structural or functional protein domains. Its better to use the whole sequence search (rather than sliding window search). Any other alternative bioinformatics approaches are not validated and might be purely exploratory in nature.	Of all the database, the COMPARE database is the most updated, comprehensive, regularly curated, devoid of false entries and accepted industry wide.	Current bioinformatics based approach is the best initial approach to predict allergenicity. The bioinformatics data combined with weight of the evidence approach is strong enough to predict possible allergenicity. Any in-vitro/in-vivo allergenicity prediction methods are prone to false positives and are difficult to validate. Allergens (unlike toxins) have differential sensitization based on the genetics and environment of the patient. So even if the tests are predictive enough, the inherent diversity in the reactivity of the patients make it difficult to implement. Food matrices and digestibility varies for each individual based on age, sex, health	Molecular stability parameters (apart from heat stability) dont add much to the understanding of allergenicity. There are known allergens which are enzyme digestion or pH stable or labile. The effect of food processing is hard to guage owing to the complex food matrices and other variables from patient to patient.	Human sera studies should only be implemented in an extreme case scenario as mentioned above with precaution. Although confirmatory, many a times they are know to have false positives.	Any available methods to predict allergenicity so far are merely exploratory in nature. Unless the methods are validated with negligible amount of false positives, they should not be used for safety assessments. Instead, the weight-of-evidence (WoE) approach should be used where the source organism, history of safe use (HOSU), bioinformatics, expression levels, heat stability are taken into consideration to establish safety.	In silico and in vitro assays should only be used if they are validated and are giving very few false positives,	Carry out ring trials (first with academicians to make sure the method is valid) and then with industry to make sure it could be implemented in a GLP/ISO or regulated setting.	Post-market monitoring tools could be a powerful tool if the monitoring method is tested, validated and then applied.	Again, its very hard to do so since each protein can cause allergenicity at different levels. Also, each patient can react differently to an allergen. Allergen avoidance might be a better solution to answer the safety concerns as allergens are mostly present in innocuous foods consumed by the whole population but only a small percentage of population is atopic.
49	scientific reprots	-	-	antibody	-	-	ELISA methods	-	-	-	-	
50	A statistical analysis will help which allergens are more important for consideration	There is only a supplementary approach- an approved clinical trial with human subjects (skin sensitization only - oral intake must not be included in human trials)	I have used only allergen online and it works well. Very user friendly.	Individual proteins are good. Would like to know how predictions can be made for extracts or whole food matrices	All	Can't answer	Both	That should be the first step and integration should be done before animal testing and clinical trials	All	Consumer feedback can be a good tool	Need to know more	
51	I don't know	I don't know	if the number of possible potential allergens is not known, it is impossible to interpret a negative result, since the probability of error in the conclusion cannot be assessed.	what people and other animals ingest is not purified protein but mixed and mostly cooked food. An isolated protein and, moreover, produced by a bacterium (no or little glycosylation in particular) is not representative of reality.	all of them	I don't know	there are no effective method to predict llergenicity. Both sensitisation and elicitation are important;	the question does not make much sense. Digestibility tests are worthless and it is impossible to know in what proportion allergenic sequences can be detected...	...	of course yes !!!	acceptability is a political problem, not a scientific one.	
52	Data regarding cross-reactivity of some known allergens (i.e. seafood antigens) could be of interest for some novel proteins (i.e. insect allergens). However, a lot remain unknown for the sensitization to classical allergens, and we have to keep investigating to have enough knowledge that allow to predict sensitization to new source of proteins.	N/A	I think they are useful for the determination of B cell epitopes. However, most the epitopes of T cells remain unknown.	All of them are necessary, but it essential to determine first which are the allergenic proteins for being able to determine later their potential interaction with the matrix and the effects of processing in their allergenicity.	Many of these parameters have been related to main characteristics of food antigens, however they are not key characteristics. A combined strategy that combine these properties and immune reactivity is the best option to assess allergenicity.	N/A	All should be taken in account.	N/A	Many in vitro methods have been reported, but the scientific community must now select which are the best ones.	A post-market strategy should be set up for the management of allergenicity of foods.	A closer monitoring for food allergenicity should be put in place	