European Food Safety Authority

GMO UNIT

Briefing notes

Technical meeting
"*Draft guidance on the allergenicity assessment GM plants*"

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Parma, Italy

European Food Safety Authority (EFSA)
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Disclaimer

The briefing notes have been prepared by the EFSA GMO Unit, in close collaboration with the EFSA GMO Panel Working Group following this guidance development, to provide background information to the participants, and to facilitate an exchange of views and expertise during the technical meeting. They are not designed to compile all comments received during the public consultation, or to review in detail each single received comment. The briefing notes explore options to revise the draft guidance on allergenicity assessment of GM plants, but do not constitute an endorsement by EFSA and its GMO Panel for their implementation at present. EFSA assumes no responsibility or liability for any errors or inaccuracies that may appear.

1. Introduction

The EFSA GMO Panel, together with a designated EFSA GMO Working Group, has recently drafted a guidance document on the allergenicity assessment of genetically modified (GM) plants. In the course of the "Open EFSA" initiative, EFSA involved and benefited from the input of scientific experts and stakeholders during an initial workshop in June 2015[1] to collect relevant information and insights for the development of the guidance at the initial stage. In a pilot "Focus Group" project of EFSA[2], Member State representatives as well as members of industries and patient organisations closely followed the development of the guidance document and actively participated to the discussions. A two months online public consultation[3] was launched on July 26th 2016 to collect input from scientific experts and stakeholders, and a total of approximately 200 comments were received.

EFSA is now organising a technical meeting to introduce the comments received during the public consultation period and to discuss them with scientists and interested stakeholders. The aim of the meeting is an open dialogue on the issues raised, and collecting further ideas on how to address these issues. The meeting is structured to provide insight into EFSA's view from a regulatory angle focusing on the scientific rationale of all relevant topics addressed in the draft guidance document. Representatives of stakeholder's groups have been selected together with the "Focus Group" to provide key aspects from the point of view of the organisations that they represent. A plenary discussion for each of the topics addressed in the draft guidance document will follow with all participants.

Briefing notes are shared with all participants prior to the technical meeting to allow an interactive exchange of views and expertise during the discussions. After the technical meeting, a summary of the discussions as well as the presentations held during the meeting will be published on the EFSA website.

EFSA aims to ensure full consideration of all received comments and stakeholders' views before the final adoption of the guidance on allergenicity assessment of GM plants, which is foreseen in spring 2017.

2. Objectives of the draft guidance on allergenicity assessment

The overall objective of the guidance is a comprehensive and harmonised approach for the allergenicity assessment of genetically modified (GM) plants, which incorporates new developments in the area. Furthermore it is the aim to assist risk assessors as well as applicants in the generation, presentation and evaluation of the requested data.

Literature reviews commissioned as well as conducted by EFSA have identified the progress in science regarding *in vitro* protein digestibility and non-IgE mediated adverse immune reactions to foods. Furthermore, novel methodologies were identified, as well as the need for better standardisation and harmonisation of the allergenicity assessment. In the light of the most recent implementing regulation EU 503/2013, the measurement of relevant individual

[1] http://www.efsa.europa.eu/en/events/event/150617
[2] http://www.efsa.europa.eu/en/stakeholder/consultativegroups
[3] http://www.efsa.europa.eu/en/press/news/160726

allergens is a mandatory requirement as part of the comparative approach. The draft guidance describes the scientific rationale and the up-to-date methodology to assist applicants and risk assessors in fulfilling these novel data requirements.

3. Revision of the draft guidance on allergenicity assessment – proposed way forward

3.1 Overall aspects

Compliance of the guidance with Codex Alimentarius

Comments: Some stakeholders commented that some tests and strategies proposed by EFSA are not in line with Codex Alimentarius[4] recommendations and the weight-of-evidence approach.

Clarification: Codex Alimentarius recommends an integrated, stepwise, case-by-case approach for the allergenicity risk assessment, which takes the evidence of several types of information into account. The current EFSA guidance does not question these principles for the allergenicity assessment.

Proposal: To better clarify that evidence for allergenicity should be collected in a stepwise, weight-of-evidence-based approach, as recommended by Codex. EFSA is in line with the principles described by Codex and it will be highlighted in the EFSA draft guidance document.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion of the individual topics.

Overall format of the guidance and lack of clarity of the proposals

Comments: Based on the received comments, some stakeholders do not agree with the overall format of the draft guidance, since it is thought to include too much background information and too little actual guidance on how to perform and interpret the data of the allergenicity assessment.

Clarification: In the view of EFSA, the background information was necessary for the public consultation period, especially for topics that have not been addressed previously, like non-IgE mediated adverse immune reactions or the proposed novel approach for *in vitro* protein digestibility.

Proposal: To move background information into the annex for the final version of the guidance, if not absolutely necessary for understanding of the document. To provide more guidance and explanation of the proposed tests in the document as considered appropriate.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion of the individual topics.

[4] Codex Alimentarius, 2009. Foods Derived from Modern Biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome.

Allergic sensitisation vs. elicitation of allergic reactions

Comments: There seems to be a lack of understanding concerning allergic sensitisation and elicitation of reactions. Based on the comments received, it is not clear which parts of the guidance refer to sensitisation and elicitation, respectively.

Clarification: Even though elicitation is the main hazard which should be assessed, the risk for novel/enhanced potential for allergic sensitisation should also be considered during the risk assessment process.

Proposal: To better clarify the connection between the proposed tests and both, allergic sensitisation and elicitation, in the final guidance document.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion of the individual topics.

3.2 *In vitro* protein digestibility

Suggested tests not in line with Codex Alimentarius

Comments: Some stakeholders commented that the proposed new conditions for *in vitro* protein digestibility are not in line with the international principles of Codex Alimentarius, and are driven by scientific curiosity.

Clarification: According to Codex Alimentarius, alternative protocols to the pepsin resistance test may be used if adequate justification is provided. It is also recognised by EU Implementing Regulation 503/2013 that the pepsin resistance test may not reflect physiological conditions and different conditions may be used.

Proposal: To discuss conditions suggested by available protocols (Thomas 2004, Mandalari 2008, Mandalari 2009, Infogest) with stakeholders in the EFSA technical meeting regarding possible conditions (pH values, enzyme concentrations, etc.) to be tested during the interim phase for *in vitro* protein digestibility. To assess useful conditions for an enhanced *in vitro* protein digestibility assessment during an interim phase, as already suggested in the draft guidance. The outcome of the discussions in the stakeholders' EFSA technical meeting could be taken into account to define these conditions.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Protein source to be used

Comments: According to the stakeholders' comments, the scientific rationale for preferring protein isolated from edible plant tissue or plant protein in general to recombinant protein expressed in bacteria is lacking in the draft guidance.

Clarification: The protein expressed in the edible plant tissue reflects the protein that is eaten and is therefore preferable to recombinantly expressed protein, if sufficient quality and quantity of the protein is available. However, if the conditions above cannot be met, recombinant protein expressed in heterologous systems can be used, provided that equivalence can be demonstrated. It is noted that proteins expressed in plant systems are qualitatively superior to proteins expressed in prokaryotic systems, since better equivalence including folding and post-translational modifications can be achieved.

Proposal: To better clarify the scientific rationale why proteins from the edible tissue or plant expressed proteins in general are preferred. To better link it to availability of sufficient quality and quantity of the appropriate protein to be tested.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Predictive/biological value of the proposed tests

Comments: Based on the received comments, some stakeholders are not sure of the biological or predictive value for allergenicity assessment gained by the proposed test conditions.

Clarification: The aim of the EFSA's proposal is to reduce the uncertainty of the allergenicity assessment by testing the *in vitro* protein digestibility of newly expressed proteins under an enhanced *in vitro* digestibility test. Therefore, it is not the aim of the proposed *in vitro* protein digestibility tests to be used in isolation to predict allergenicity, since this is currently not possible using a single test. The conditions suggested should better reflect the ones actually found in consumers, as suggested by EU IR 503/2013[5], including conditions for infants and others at risk for allergy.

Proposal: To better clarify that the purpose of the test is not to be used in isolation for the prediction of allergy, but to reduce the inherent uncertainty of the allergenicity assessment of newly expressed proteins. An interim phase is suggested to evaluate the efficacy of the proposed revisions to the *in vitro* gastrointestinal digestion test.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Rationale for 9 amino acid cut-off

Comments: Stakeholders expressed their concern regarding the 9 amino acid cut-off, for which only insufficient scientific rationale is provided, especially for IgE-mediated allergic reactions.

Clarification: The size cut-off is clearly defined and scientifically justified by the minimum peptide length necessary for HLA binding and T cell activation. It is mentioned in the guidance that the minimal size for IgE-mediated reactions and antibody cross-linking is less clear, and might be more than 9 amino acids.

Proposal: To keep the 9 amino acid cut-off in order to harmonise the approaches for risk assessment. However, additional discussion is needed to understand how this cut-off should be interpreted in the case of IgE-mediated adverse reactions. To acknowledge that the risk of adverse reactions is lower, the smaller the fragments are after *in vitro* digestion.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Effects of the food matrix

Comments: Some stakeholders expressed their concern that the food matrix is not taken into account in the proposed new *in vitro* protein digestibility tests.

[5] EC, 2013. Commission Implementing Regulation (EU) No. 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No. 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No. 641/2004 and (EC) No. 1981/2006. Off. J. Eur. Union L157, 1–48.

Clarification: It is acknowledged in the draft guidance document that the food matrix might have an influence on digestibility of proteins. However, because of the diversity of possible food matrices and food processing procedures, our knowledge on their effects on protein degradation is limited and therefore difficult to predict.

Proposal: To further elaborate the aspects described above.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

3.3 Endogenous allergenicity

Assessment/Measurement of endogenous allergenicity

Comments: The usefulness of the assessment of endogenous allergenicity in GM crops was challenged by some stakeholders.

Clarification: The measurement of allergenicity as part of the compositional analysis is mandatory with the EU IR 503/2013. Furthermore, allergens are mentioned in the context of key components to be considered following a comparative assessment by Codex Alimentarius.

In the case of soybean, anaphylactic reactions are known, especially in children. Low prevalence does not preclude EFSA to protect a small percentage of people. Soybean is recognised as a common allergenic food by EU Regulation[6,7].

Proposal: The comparative assessment of endogenous allergens is considered useful and in such respect recommended by Codex Alimentarius, EFSA guidance documents and IR 503/2013. Therefore, no deviations from the main principles described in these guidance documents and international agreed standards are expected at this point in time.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Data interpretation and natural variability

Comments: Based on the comments received, stakeholders believe that the natural variability is a crucial parameter to interpret the data assessed on endogenous allergen expression levels. There is concern that it is not possible to take the full range of natural variability into account. Stakeholders also commented that the scientific literature should be used as a basis to consider natural variability.

Clarification: The consideration of natural variability for data interpretation is already one of the key aspects described in the draft guidance. It is noted that the absolute safety of products cannot be assessed. EFSA has developed a field trial design for the comparative compositional analysis which does not rely on baseline data from databases[8,9]. EFSA agrees with stakeholders that data from scientific literature could be used as a basis for the data interpretation in the future, as suggested in the guidance.

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[6] EC, 2003. Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs.

[7] EC, 2011. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[8] EFSA, 2010b. EFSA panel on genetically modified organisms (GMO). Scientific opinion on statistical considerations for the safety evaluation of GMOs. EFSA J. 8 (1), 1250.

[9] EFSA, 2011a. EFSA panel on genetically modified organisms (GMO). Guidance for risk assessment of food and feed from genetically modified plants. EFSA J. 9 (5), 2150.

Proposal: To clarify that the term "natural variability" and its interpretation concerning the evolution of cultivars to be produced and commercialised. EFSA encourages the standardisation and harmonisation of the methods used to enhance measurement comparability and the possible future establishment of an allergen database, as described in the draft guidance. To this end, cooperation regarding data sharing especially between EFSA and applicants is necessary.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Selection of soybean allergens to be measured

Comments: Based on the comments received, some stakeholders criticised the number of soybean allergens to be measured as described in table C1 of the document. There seems to be a lack of clarity, how to justify the measurement of allergens by "scientific rationale".

Clarification: Table C1 is a collection of data on potential soybean allergens available from the scientific literature, and should not be understood as a mandatory list of proteins to be measured. Indeed, a strategy how to actually choose proteins out of the ones described in table C1 is described in the draft guidance. It is the aim of the guidance to provide a possible strategy for selecting proteins to be measured which can also be applicable to other foods than soybean. Indeed, clinical relevance of a potential allergenic protein should be the main concern and argument to include an allergenic protein in the assessment.

Proposal: To clarify that table C1 is not a list of allergens to be measured, but an example on how potential allergens could be identified, which are, in the next step, evaluated to choose the ones to be measured. To better clarify that international recognition of allergens and their clinical relevance should be the main reasons and concerns leading to inclusion of a protein into the measurement of endogenous allergenicity. A refined strategy for selecting possible allergens to be measured should be discussed during the meeting and with clinical experts.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Exposure – sensitisation – allergen thresholds

Comments: Some stakeholders suggested deleting the reference to allergic sensitisation in the context of enhanced expression of endogenous allergens. It was criticised that the connection between amount of allergens and sensitisation has not been shown, or that there are no thresholds in place for sensitisation.

Other stakeholders wanted clarification on the interpretation of the term "safe level".

Clarification: The current focus of the draft guidance is indeed on elicitation, since more data is available in this respect. However, allergic sensitisation might still be a factor of allergen levels and exposure, and the possible risk of novel sensitisations in connection with allergen levels cannot be ignored.

EFSA acknowledges the fact that a "safe level" for allergen expression is nearly impossible to state. However, it is the requirement that GM crops are as safe as conventional crops, which is achieved by the comparative approach.

Proposal: To clarify that, even though data showing a connection between allergen dosage and allergic sensitisation are scarce, allergic sensitisation might still be a factor of allergen levels and exposure.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Methodology

Comments: Some stakeholders had the impression that EFSA prefers mass spectrometry-based methodology over ELISA for the assessment of endogenous allergens.

Clarification: Mass spectrometry and ELISA are equally acceptable methodologies for the assessment of endogenous allergenicity, if the considerations for each method are taken into account, as described in the current draft guidance.

Proposal: To clarify that both methodologies, as suggested in the draft guidance, are currently equally acceptable for the measurement of endogenous allergens.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Other plants containing allergens apart from soybean

Comments: Some stakeholders expressed their concern that, apart from soybean, endogenous allergenicity is not assessed in other crops like for example maize, which also contains potential allergens.

Clarification: Maize is currently not considered as a common allergenic food by European Regulation; therefore the assessment is not mandatory. However, the guidance and the example provided in the EFSA document on how to identify allergens and interpret results may be used for other crops in the future, if necessary.

Proposal: To better clarify that the current guidance may be applied to other crops than soybean in the future, if considered necessary and appropriate. To this end, risk assessors, risk managers, health professional and stakeholders can provide invaluable feedback.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

3.4 Non-IgE mediated adverse immune reactions to foods

Proteins to be assessed

Comments: Based on the comments received, there seems to be a lack of clarity on which proteins should be assessed in the course of the risk assessment for celiac disease.

Clarification: Risk assessment cannot be limited to proteins from sources which are known to contain proteins toxic for celiac disease patients. The risk assessment should be provided for any protein for which no knowledge on exposure is available. The state of the art in science has moved on to a risk assessment that can be based on the underlying molecular mechanisms of disease.

Proposal: To provide more clarity and guidance concerning the proteins to be assessed in the risk assessment for celiac disease. The necessity of experimental data might be discussed for proteins for which safe consumption by celiac disease patients is well documented.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Stepwise approach for the proposed methodology

Comments: It seems to be unclear for several stakeholders, how the stepwise approach for celiac disease risk assessment is to be implemented.

Clarification: Based on the current state of scientific knowledge in the area, the stepwise approach proposed provides the possibility to stop the risk assessment process at the *in silico* step if no hazards are identified and no further *in vitro* tests are necessary. If a hazard cannot be excluded in the first *in silico* steps, the more laborious *in vitro* tests should be taken into account according to the weight-of-evidence approach.

Proposal: To be clarified by EFSA that not the whole cascade of suggested tests is necessary for the safety assessment, if no hazard is identified by *in silico* testing.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.

Standardisation and validation of the proposed methodology

Comments: Some stakeholders raised concern because of a lack of standardisation and validation of the proposed methodologies and tests suggested in the risk assessment for celiac disease.

Clarification: The proposed tests and methodologies are following the current state-of-the-art in science for celiac disease. These methods are the best available approaches EFSA could identify to minimise the risk for celiac disease patients consuming food altered by biotechnology. The proposed tests are highly relevant since toxic proteins display unique features and HLA-DQ binding and subsequent T cell recognition are crucial for toxicity.

Proposal: To better explain the methodological proposals in depth when necessary and to provide example approaches with the aim to allow a better standardisation of dossiers.

Discussion: Stakeholders are invited to discuss this point during the plenary discussion.