EFSA GMO Network - SUBGROUP ON NGTs
Minutes of the 1st meeting

29 May 2024
14:00-18:00 (CET)
Minutes agreed on 5 July 2024

Location: Sciensano (Eurostation), Ernest Blerotstraat/Rue Ernest Blerot 1 1070 Brussels

Attendees:

- Subgroup Participants:

Country	Organisation
Austria	Environment Protection Agency
Austria	AGES
Belgium	Flanders Institute for Biotechnology (VIB)
Belgium	Sciensano
Croatia	Faculty for Natural Science and Mathematics
Cyprus	Agricultural Research Institute (ARI)
Czech Republic	Charles University
Denmark	DTU Food
Finland	Finnish Food Authority
France	ANSES
Germany	Federal Agency for Nature Conservation
Germany	Federal Office of Consumer Protection and Food Safety (BVL)
Hungary	Ministry of Agriculture
Ireland	Food Safety Authority of Ireland (FSAI)
Italy	Istituto Superiore di Sanità (ISS)
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Netherlands	RIVM
Netherlands	Wageningen Food Safety Research (WFSR)
Norway	Norwegian Scientific Committee for Food and Environment
Poland	Institute of Human Genetics Polish Academy of Sciences
Poland	Plant Breeding and Acclimatization Institute - National Research Institute
Romania	University of Life Sciences "King Mihai I" Timisoara
Romania	University of Agronomic Sciences and Veterinary Medicine of Bucharest
Spain	Ministry for Ecological Transition and the Demographic Challenge
Spain	Polytechnic University of Valencia
Sweden	The Swedish Board of Agriculture

- Hearing Experts:
  Ewen Mullins, Fabien Nogué

- European Commission/Other EU Agencies representatives:
  None
- EFSA:
  NIF Unit: Nikoletta Papadopoulou (Chair), Tommaso Raffaello, Reinhilde Schoonjans.

1. Welcome and apologies for absence

The Chair welcomed the participants. Apologies were received by Norway who appointed an alternate participant.

2. Adoption of agenda

The agenda was adopted without changes.

3. Terms of reference (ToR) and Objectives of the Subgroup on NGTs

Abstract

EFSA introduced the Terms of Reference (ToR) and the overall objectives of the Subgroup on NGTs. The establishment of the Subgroup on NGTs was approved by the EFSA Advisory Forum (AF) in March 2024. The AF consists of representatives from 27 EU Member States, Iceland, Norway, Switzerland, seven pre-accession countries, and the European Commission as observers. Its primary functions include advising EFSA on work programs and priorities, ensuring collaboration between national bodies and EFSA, resolving contentious scientific issues, avoiding duplication of efforts, and increasing scientific cooperation.

The subgroup's main objective is to foster knowledge sharing on NGTs, including their development and application to plants, animals, and microorganisms, increase preparedness, and jointly address potential future RA challenges.

Members should have expertise in NGTs and experience in molecular characterization, food and feed, and/or environmental RA of GMOs. Member States can appoint one participant and one alternate. The subgroup will meet at least once a year, either physically or virtually, and the working language will be English.

EFSA informed that the Subgroup on NGTs is currently composed by 30 participants and alternate from 19 Member States, while 9 Member States have not nominated any expert. The proceedings of the subgroup's meetings will be documented and published on the EFSA website.

Discussion

France (ANSES) requested clarification on the impact of the decision to implement the proposed EC draft regulation on the risk assessment of NGT plants, to the scope of this subgroup. EFSA confirmed that discussion on the EC proposed criteria for Category 1 NGTs and equivalency to conventional, as adopted on the 5thJuly 2023, is not in the remit of the subgroup on NGTs. The subgroup rather focusses on the scientific rationales for assessing risks by NGT plants that are proposed to remain under the GMO regulation ('Category 2 NGT plants' or similar). Therefore, EFSA

considered it important to formalize the Subgroup on NGTs in order to share knowledge regarding RA of gene edited organisms; regulatory changes will also be considered according to the developments.

France (ANSES) asked what type of interaction with the GMO Panel is foreseen. EFSA clarified the Subgroup on NGTs is to be considered a consultive body while the EFSA GMO panel independence is maintained. The knowledge of the group of experts participating to this group can be leveraged to provide further input to the GMO Panel activities on NGTs. There are currently no concrete mandates for such activities. EFSA emphasized that the need to discuss risk assessment aspects of NGT plants is independent from the fate of the EC proposal since standard GMO applications may already contain characteristics of NGTs that would deserve further discussion.

Germany (Federal Agency for Nature Conservation) commented on the process of drafting the ToR and the nomination of the participants and alternates from the AF, noting that the short timeline may have caused some confusion. Also, Germany noted that the number of participants and alternates of the subgroup is higher than what is indicated in the ToRs. EFSA explained that modifications were made during the process and that the establishment of the subgroup had to follow the same procedural steps by the AF as for any other Network in EFSA.

4. Tour de table

The participants and alternate participants introduced themselves. A Word file was circulated after the meeting where the participants and alternate members of the Subgroup on NGTs can add information about their professional experience which is relevant for this subgroup remit.

5. Introduction of the case studies and RA methodologies

Abstract

EFSA introduced the exercise which aimed to discuss risk assessment (RA) methodologies for plants developed using NGTs and identify areas needing further discussion or development.

EFSA presented the published criteria for the RA of NGT plants (EFSA statement available here) which focused on evaluating the presence of exogenous DNA, the source of new genes, whether integration is random or targeted, potential gene interruption, the history of safe use, and the function and structure of the inserted genes.

Two case studies were presented to illustrate different RA challenges for potential NGT applications under the current GMO Regulation:

Case study 1: the first case study involved common wheat with reduced α-gliadin content achieved through a CRISPR/Cas9 construct targeting over 30 Gli-2 loci. The CRISPR/Cas9 cassette will not be present in the final wheat product. The scope was limited to import and processing.

Case study 2: the second case study focused on durum wheat engineered for leaf rust (Lr) resistance. This was achieved by replacing endogenous Lr genes with genes from a wild relative using CRISPR/Cas9.

Additionally, 15 susceptibility genes were disrupted to promote durable resistance. Similar to the first case study, the CRISPR/Cas9 cassette would not be present in the final product. The scope was also limited to import and processing.

The current GMO RA Requirements in four main areas: Molecular Characterization (MC), Comparative Analysis (CompERA), Food/Feed Risk Assessment (FF), and Environmental Risk Assessment (ERA) were presented (see **Annex I**).

EFSA highlighted some of the main points raised in the EFSA criteria document such as the issue of the 'single first principle' in case of high number of inserted/modified sequences, the assessment of multiple novel proteins in case of high number of modifications/challenging for protein characterization-assessment, the History of Safe Use/Familiarity with the environment concepts and the comparative approach for genome-edited plants with complex trait.
Three groups were formed, 2 in the meeting room covering case study 1 and 2 (6 participants each) and 1 for the on-line attendances covering case study 2.
The participants were asked to focus on 2 questions:

What are the RA challenges for MC, COMPERA and FF areas using current RA requirements? and

What are the RA areas that need further development and/or elaboration when assessing NGT plants?

The groups' discussions are summarized under Item 7.

Discussion

Participants requested some clarifications on the presentation and the group discussion. The Netherlands (RIVM) asked whether the introduced genes in case study 2 would be cisgenic or intragenic. EFSA clarified that, being a hypothetical case, both conditions could be assessed in the group discussion. Germany (Federal Office of Consumer Protection and Food Safety) noted that Annex II of the EC proposal sets the framework for the whole process of proportionate case-specific risk assessment. EFSA clarified that the published EFSA criteria could be used as starting point for the group discussion and, if time allows, a comparison with what is included in Annex II of the EC proposal can also be discussed. The Netherlands (Wageningen Food Safety Research) reflected on the possibility that certain effects (i.e. autoimmunity in the modified NGT plant) may not be predicted from the molecular data, but rather from the assessment of the agronomic and phenotypic properties of the NGT plant. EFSA replied that certain unwanted characteristics may probably be eliminated during the development of the NGT plants (e.g. by backcrossing). Belgium (Sciensano) requested clarification on how the case studies were used by the GMO Panel when reflecting on the risk assessment of NGT plants. EFSA clarified that GMO Panel used case studies to develop and test the proposed criteria for the proportional risk assessment of NGT plants. EFSA also emphasized that at the time of the publication of the EFSA criteria, the EC proposal and the 2 categories for NGT plants had not been developed yet. EFSA also clarified that the 2 case studies were used to verify whether or not the different current requirements for the risk assessment of GM plants would still be applicable for the risk assessment of NGT plants. Poland (Plant Breeding and Acclimatization Institute - National Research Institute) reflected on the criteria of history of safe use (HoSU) and whether it should be applied to the product

or to the technique used for the genetic modification. EFSA clarified that HoSU is one of the criteria to be used for the proportionate risk assessment but the definition of HoSU would need further discussion and elaboration, as explained in the EFSA statement on RA criteria for NGT plants. Poland agrees that its definition should be better clarified before this criterion can be applied. EFSA noted that the term HoSU is also used in the Novel Food regulation. The Netherlands (Wageningen Food Safety Research) reflects on the HoSU of the modification/mutation and previous population exposure and whether such mutation can be achieved via conventional breeding techniques. In response to previous comments from Poland, Belgium (Flanders Institue for Biotechnology) clarified that the interpretation of the regulation (recital 17 of Dir 2001/18/EC) from a legal perspective is that HoSU is more related to the techniques, rather than the product.

The group discussion which followed the introduction of the case studies is reported below in Item 7.

7. Discussion and identification of areas needing clarifications and future tasks of the subgroup

Following the discussion within each group, the participants reconvened to summarize the main points. It should be noted that that the intention of the discussion in Item 7 was to brainstorm on challenges and data requirements for RA, whose main points are summarized below, without coming to a consensus. Therefore, the summary below highlights the main points of the discussion and does not necessarily reflect the opinion of each participant. Due to lack of time, question 2 (i.e. *What are the RA areas that need further development and/or elaboration when assessing NGT plants?*) was not fully addressed.

Case study 1 ('low gluten wheat', see description in Item 5)

The participants considered that the description of the methods used for the genetic modification would be needed in this case. Regarding the nature and source of the vector used, the participants considered that this would also be needed. However, in case the plant is granted already an "NGT status" (i.e. it is non-transgenic) by another authority for example, less information would be needed in this case.
Other information such as information on the donor organism(s), copy number and size of all detectable inserts, information on the nucleic acid(s) sequence(s) intended to be inserted, flanking sequences, open reading frames (ORFs) in flanks, ORFs in the insert, genetic stability and homologous recombination/horizontal gene transfer (HGT) would not be needed given the absence of any insert.
The description of the introduced trait(s), of the resulting changes on phenotype and metabolism of the GM plant would be needed in this case. Regarding the information on the sequences actually inserted/deleted, the participants considered that in this case precise description of the edited loci would be needed. The level of analysis of the consequences of the editing and off-target analysis were also discussed.
The participants also discussed the need of protein expression analysis. For this case study, the editing is supposed to knock-out target genes, therefore some participants considered that the quantification of the expression level(s) would not be needed, and the analysis should focus on the end result of the editing that is level of gliadins. Some participants proposed that information would be needed only for editing leading to gain of function and not for editing leading to loss of function. Other views

considered that for some of the genomic edits the absence of the protein should be demonstrated. In this case there should be a hypothesis on why the editing could lead to incomplete knock-out (for example, the possibility of alternative splicing).
Although there are no newly expressed proteins in this case study, some participants considered that there could be the possibility that a frame-shift would lead to a new ORF. The need to further analyse these potential new ORFs was discussed. Similarly, some participants considered that in case there is doubt on the knock-out status of one of the targets (possibility of alternative splicing for example) information for RA could be needed. Regarding the search for similarity to toxins and celiac analysis, the same discussion as for the ORFs above may apply.
The participants considered that the comparative approach remains a cornerstone. This method is deemed crucial for evaluating the safety and impact of genetically modified plants. For this case study, potential comparators were identified, including conventional non-modified wheat and wheat varieties with lower gluten content. The selection of the appropriate comparator hinges on the specific question that should be addressed. Historically, for transgenic plants, comparative assessments have served as the foundation to pinpoint issues warranting deeper investigation. However, the group discussed a possible deviation from this traditional approach for this case study. Some participants suggested that while comparative assessment is still valuable, it should be adapted to better fit the different products. When it comes to data requirements, if field trial data are necessary to test a risk hypothesis, the existing data requirements will generally apply. However, participants discussed some exceptions: agro/phenotypic and germination data may not be required in this instance, as this case study does not involve the cultivation of the plants. Additionally, there was a discussion on whether the field trial design should mimic that used for transgenic plants, in terms of replications and number of reference variety lines. It was suggested that the trial design could be more targeted, tailored specifically to the RA questions to be addressed.
Regarding the discussion on the food and feed assessment, the participants found it scientifically inconsistent to require an assessment under the GMO legislation for a plant with 30 modified gluten genes but – in the case of Category 1 NGTs- not for those plants with 20 genetic modifications in modified gluten genes. However, the participants acknowledged that the categorization of the plant will depend on the final format of the regulation following the EC proposal.
In terms of data requirements for this assessment, the participants noted several key points. Protein characterization should focus on the end product rather than the specific proteins. However, identifying the proteins produced remains important. For most participants, the 90-day study is only relevant if there is a specific risk hypothesis that needs testing. Additionally, it may be necessary to test for endogenous allergens to determine if changes in gluten content affect or stimulate their production.
The participants considered that several data requirements are not relevant for this case. These include the protein characterisation and equivalence, as no new proteins are introduced in the plant, but rather endogenous protein function is depleted. There is no assessment of equivalence between plant and recombinant proteins. Similarly, *in vitro* stability tests, which assess the stability of new proteins, are not needed since no new protein is produced. Finally, the 28-day study is also considered not needed, given the absence of any specific new protein expressed.

Case study 2 ('leaf rust resistant wheat', see description in Item 5)

Regarding the method of editing, participants noted that instead of using transient integration of DNA to express the CRISPR-Cas gene editing machinery, ribonucleoproteins could also be utilized. This method would eliminate the need to check for the integration of foreign DNA (transgenes). On the gene interruption and physiological effects, more detailed information is needed on the genes being interrupted, as resistant plants can exhibit specific physiological effects. Participants noted that information on the methods of development and molecular characterization is essential. Initially, assessments should be conducted on a case-by-case basis and/or to the same level as currently done for GM plants obtained via established genomic techniques, with progressively relaxed requirements as familiarity with the technology increases over time (e.g. after 10 years experience).
Regarding the molecular characterization, several factors must be considered such as the size of the modified sequence. For example, a larger insertion does not necessarily mean a higher risk but at the same time even a single amino acid change can have a significant impact. Regarding the targeted introduction of cisgenes, participants noted that data on the insertion locus is crucial and the final product should be homozygous. Longer insertions in coding regions could also impact allergenicity characteristics of the plant. Participants also considered that gene replacement may be complex, especially when multiple genes are replaced either successively or simultaneously. It is also essential to verify that there are no changes to the sequences introduced. The participants also proposed a new category of NGT plants containing only knock-out genes and sequences already present in nature or in existing food crop varieties for which no risk assessment would be necessary. In addition, when many alleles are being modified simultaneously, segregation may yield different allele combinations unless homozygosity is achieved (e.g., using double haploids).
Participants also discussed the off-target modifications which are a concern in gene editing. For example, using specific sgRNAs for each gene or a generic version for multiple genes can influence the likelihood of off-target mutations. Techniques based on homologous recombination should be preferred to reduce undesired modifications. However, some participants commented that off-targets analysis may not be relevant in case the plant phenotype does not show alteration and also the frequency of these mutations is lower than in conventionally-bred plants. Moreover, the number of off-targets which are present just after the genetic modification step(s) may be higher than the number of off-targets remaining after the backcrossing during the breeding process for variety development. For the identification of off-targets, whole-genome sequencing (WGS) is preferred over bioinformatics prediction tools, as prediction tools trained on animal models may not reliably detect plant off-targets. Combining both methods, using bioinformatics predictions to guide focused sequencing, can be effective. However, some participants argued that WGS may not be ideal to confirm small nucleotide changes.
Protein characterization should distinguish between knock-out proteins, which can occur naturally, and genes from the breeders' gene pool. Participants noted that protein characterization would not be required for knock-out proteins. Since endogenous allergens are a concern, particularly in wheat, the allergenicity of modified proteins must be evaluated to determine if their allergenicity properties have changed. Bioinformatics can be sufficient for this assessment, but there is a

need to consider whether edited endogenous gene sequences could become allergenic.
Participants noted that the scenario involving the simultaneous modification of many genes is similar to novel food risk assessment with numerous new proteins. Current approaches for assessment of toxicity and allergenicity, which test each single component fully, are inadequate for assessing the multitude of new metabolites and proteins in such cases. Knock-outs where a protein is deleted are less concerning, but the closeness of genes from wild relatives to the host plant must be considered.
Regarding the comparative assessment, a case-by-case approach is recommended, with requirements for certain aspects. If a genetically modified plant is comparable to conventional food and no significant changes are identified, further assessment may not be necessary.
The participants did not identify specific issues concerning the environmental risk assessment.
Some additional notes from participants suggested that knock-outs and mutations resulting in protein sequences already occurring in the breeders' gene pool should be considered as safe as those introduced by conventional breeding. However, longer insertions may alter allergenic potential, requiring risk assessment.
Moreover, the phenomenon of "hybrid necrosis" in wheat Lr genes was highlighted, where young F1 plants die due to an autoimmune response when crossed with an incompatible variety. However, this effect would typically be detected during conventional breeding and variety registration.

8. End of the meeting

The Chair thanked the participants for their active participation and the fruitful discussion. The draft minutes will be shared with the participants and published on the EFSA website together with the presentations within 15 working days. The meeting was closed at 18:00.

Update after the meeting

A report of the Subgroup on NGTs was provided to the 17th GMO network meeting on 31st May 2024.

A survey was launched to the Subgroup on NGTs participants to provide feedback and to propose possible topics to be discussed at future meetings.

Annex I

Relevant RA requirements of the GMO regulation served as the foundation for the group discussion.

Molecular characterization

1. Description of the methods used for the genetic modification
2. Nature and source of the vector used
3. Information on the donor organism(s)
4. Information on the nucleic acid(s) sequence(s) intended to be inserted
5. Description of the introduced trait(s), of the resulting changes on phenotype and metabolism of the GM plant
6. Information on the sequences actually inserted/deleted: Sequencing package/report
7. Copy number and size of all detectable inserts
8. Flanking sequences
9. Open reading frames in flanks
    1. similarity to known allergens
    2. similarity to known toxins
10. Open reading frames in the insert
    1. similarity to known allergens
    2. similarity to known toxins
11. Protein expression study
12. NEPs
    1. sequence similarities to known allergens
    2. sequence similarities to known toxins
    3. Coeliac disease
13. Genetic stability of the inserted/modified sequence
14. Homologous recombination / Horizontal Gene Transfer (HGT)

Comparative assessment

1. Choice of the comparator
2. Field trials description (including management practices)
3. Suitability of the test materials
4. Meteorological data
5. Experimental design of the studies in support of the comparative analysis
6. Germination study
7. Statistical analysis
    1. Agro/pheno
    2. Seed
    3. Forage

Environmental risk assessment (ERA) and Post-market environmental monitoring (PMEM) plan

1. General approach
2. Persistence and invasiveness including plant-to-plant gene flow
3. Plant to microorganism gene transfer
    1. HGT BI analysis

4. Interaction between the GM plant and target organisms
5. Interaction of the GM plant with non-target organisms (NTOs)
6. Impacts of the specific cultivation, management and harvesting techniques
7. Effects on biogeochemical processes
8. Effects on human and animal health
9. PMEM Plan

Food & Feed

1. Protein characterisation (e.g. mol/biochem)
2. Physicochemical and functional equivalence
3. Protein equivalence (in case of NEPs produced in binary system)
4. Stability in-vitro (Digestibility)
5. Stability in-vitro (influence of pH and T°)
6. 90d study
7. 28d study
8. IgE binding human sera (in case of NEPs form allergenic sources)
9. Endogenous allergens
10. Human nutrition
11. Animal nutrition
12. Human dietary exposure
13. Animal dietary exposure