NETWORK ON RISK ASSESSMENT OF GMO

Minutes of the 15th meeting

8-9 June 2023
14:00-18:00 / 09:00-13:00
Minutes agreed on 3 July 2023

Location: Crop Research Institute, Prague (CZ) and Online

Chair: Ana Afonso (Head of the Nutrition and Food Innovation (NIF) Unit)

Attendees:

- Network Participants:

Country	Name
Austria	Marion Dolezel, Markus Woegerbauer
Belgium	Adinda De Schrijver
Bulgaria	Dimitar Djilianov, Tzveta Georgieva
Croatia	Renata Hanzer
Cyprus	Andre Varnava-Tello
Czech Republic	Hana Jirakova, Jaroslava Ovesna
Denmark	Agnieszka Podolska Charlery, Radhakrishna Shetty
Estonia	Triin Sellis
Finland	Annikki Welling, Kirsi Tormakangas
France	Youssef El Ouadrhiri
Germany	Wolfram Reichenbecher, Andrea Scheepers
Greece	Dionysia Stefanitsi
Hungary	Rita Andorko', Dora Tarjany
Ireland	Chloe Glennon, Bernadette Murray, Emma O'Leary, Patrick O'Mahony
Italy	Marzia De Giacomo, Elena Sturchio
Latvia	Lelde Grantina Ievina
Lithuania	Odeta Pivoriene
Luxembourg	Luc Schuler
Netherlands	Marco Gielkens, Lianne Bouwman, Gijs Kleter
Norway	Ville Erling Sipinen
Poland	Slawomir Sowa, Ryszard Somski
Romania	Raluca Mihalachioiu
Slovak Republic	Katarina Fasiangova, Zuzana Kozovska
Slovenia	Martin Batic
Spain	Carmen Cuadrado, Magdalena Ibanez Ruiz, Felix Ortego, Gema Perez Farinos
Sweden	Johan Alander, Erik Axlesson

- Observers:Mikulas Madaras (Head of the Crop Research Institute, Czech Republic, Agenda Item 1), Nur Koyuncu (Turkey)

- European Commission/Other EU Agencies representatives: Juliette-Marie Margueritte, Olga Orlova, Kathleen Lehmann

- EFSA NIF Unit: Ana Afonso (chair, Agenda Item 2), Antonio Fernandez (Agenda Item 3 and 11), Dafni Maria Kagkli (Agenda Item 4 and 11), Michele Ardizzone (Agenda Item 7), Reinhilde Schoonjans (Agenda Item 8), Aleksandra Lewandowska (Agenda Item 12)

DAY 1

Item 1: Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Sanja Milos (Croatia).

Welcome from the Head of The Crop Research Institute – CRI

The Head of the Crop Research Institute welcomed the participants.

Adoption of agenda

The agenda was adopted with changes: Belgium requested to receive information on the identification number of GMO applications/dossiers, on how to receive updates on targeted consultations and on DMS issues.

Agreement of the minutes of the 14th Network meeting held on 17-18 November 2022, via web-conference

The minutes of the 14th GMO Network meeting had been previously agreed by written procedure on 13 December 2022 and published on the EFSA website.[1]

Item 2: Risk assessment of GMOs – Partnership possibilities

Abstract

The Chair presented the partnership possibilities between EFSA and Member States according to the new opportunities envisaged by Regulation (EU) 1381/2019 (the 'Transparency Regulation'). The Chair informed the participants about EFSA contracts in place to support the risk assessment (RA) of GMOs. The Chair informed that the Transparency regulation brings the possibility for Member States organizations listed under Art. 36 of Regulation EC 178/2002 and Art. 1 of Regulation EC 2230/2004 (link) to draft RAs to be reviewed by the GMO panel for adoption. At the end of the presentation, the Chair proposed 3 questions for discussion.

Abstract presentation from EFSA

[1] https://www.efsa.europa.eu/sites/default/files/2022-12/minutes.pdf

Discussion

The Netherlands acknowledged that the possibility for a Member State to perform work related the RA of GMOs resembles the situation in place before EFSA was founded. Norway informed that a collaboration with EFSA in some food domains (i. e. food and feed additives, and food enzymes) is already in development, although not in the GMOs area yet. The Chair emphasized the eligibility criteria for any institute from any Member State to be nominated to the Art.36 list. The Netherlands informed that many Member States routinely perform the assessment of the validated dossiers, and they submit comments in the targeted consultation. In addition, it was asked whether other types of engagement would be possible also during the GMO Panel evaluation period of the applications. Austria stated that the Member State is heavily involved in the RA and asked whether this work would be eligible for refunding. The Chair clarified that work performed in the already established targeted consultation period is not to be paid, but instead different opportunities for the involvement of the Member State could be agreed, such as risk assessment in preparation of the GMO Panel review (e.g., via grant contracts). The Netherlands proposed that institutes selected to performed certain RA tasks should be clearly separated from the MS positioning on the GMO to avoid confusion. The Chair reminded the eligibility criteria which are independence and scientific expertise. Belgium requested clarification on the distinction between competent bodies and competent authorities. The Chair clarified that the discussion is focused on the competent bodies able to carry out part of the RA of the GMOs. Belgium informed that in the past, the Member State carried out environmental RA of certain GMO applications, but further internal discussion is needed to explore these new possibilities to cooperate with EFSA. Belgium also stated that differences in the RA approach between Member States and EFSA could complicate the collaboration. The Netherlands emphasized that operational challenges might limit the implementation of this type of collaboration between EFSA and Member States on the RA of GMOs.
The Chair acknowledged that despite several challenges may exist, this new collaboration on the RA of GMOs should be exploited. Latvia and Czech Republic informed that resources are currently limited and performing this preparatory work could be challenging. The Chair emphasized the aspect that this collaboration would allocate new resources and collaboration between different countries is also possible. Finland acknowledged that there are institutes at the Member State level able to carry out part of the RA and resources allocation to the Member State could facilitate this type of collaboration. Slovenia stated that human resources might also be a limiting factor for some Member States. Poland stated that there are challenges in implementing this collaboration due to divergent views/approaches on GMOs (harmonization needed) and verification on the competences would also be needed (e.g. validation of the detection methods where Poland is currently involved). Hungary suggested that separation is needed between the Member State involved in the targeted consultation of GMO dossiers and the competent body carrying out part of the RA. Czech Republic suggested EFSA to carefully reflect on how this collaboration would be implemented. The Chair emphasized that the RA of GMO is centralized in EFSA, but Member States are also carrying out RA of GMO applications for their risk management bodies. In addition, the legislator has foreseen this type of collaboration to avoid duplicating work for better use of EU resources. The Chair concluded by acknowledging challenges in the GMO areas but also by encouraging the participants to reflect on this partnership possibility by also checking the list of

competent authorities under Art. 36 (link) and informed that a discussion is currently ongoing at the Advisory Forum and EFSA Management board level.

Item 3: Protein safety of present and future GM plants

Abstract presentation from EFSA

Current requirements for the safety assessment of newly expressed proteins in plants is set by Commission Implementing Regulation (EU) No 503/2013. The main requirements are based on principles adapted from the chemical risk assessment area and on guidelines of Codex Alimentarius for the safety assessment of foods derived from 'modern' biotechnology published in 2003. Nowadays, these assessments are increasingly difficult because GM plants may contain a high number of newly expressed proteins that in some cases are also difficult to be tested, e.g. membrane proteins. The practical implementation of current international guidelines, which are mainly targeted to assess a few number of proteins, is therefore challenging. Experience gained in the assessment of regulated products and new developments in the field call for a modernization of key steps in protein safety testing. The GMO Panel aims at developing a statement reflecting on the topic and proposing new ways for the assessment of newly expressed proteins. Member State representatives were asked to comment and provide views on the safety assessment of proteins.

Abstract presentation from Germany

Regarding protein safety of present and future GM plants, Germany followed up with a presentation on upcoming challenges and (possible) questions to discuss. The presentation did not give any concrete answers yet but was mainly given to stimulate the following discussion with the long-term goal to support EFSA defining a new logic flow for a stepwise, case-by-case, weight of evidence approach in the protein safety assessment. The areas addressed included a) HoSU with questions about possible definition, concept(s) and criteria, b) questions about risk assessment of complex/complicated cases, e.g. high number of newly expressed proteins and c) questions about risk assessment while avoiding animal testing considering the 3R principle. In this context, the question was also raised as to whether or to what extent both a revision of existing methods and the development of new methods are needed. Overall, the need for a shift in experiments within the given weight-of-evidence approach seems worth discussing.

Abstract presentation from The Netherlands

In-vitro research

Role of intestinal transport of digested proteins on basophil activation

A recent publication[2] elucidates the important additional role that intestinal transport of digested allergenic food proteins potentially has besides protein digestibility itself. During such intestinal transport (*i.e.,* uptake from the intestines and transport across

[2] Smits, M., Nooijen, I., Redegeld, F., de, A., Le, T.-M., Knulst, A., Houben, G., Verhoeckx, K., Digestion and Transport across the Intestinal Epithelium Affects the Allergenicity of Ara h 1 and 3 but Not of Ara h 2 and 6. Mol. Nutr. Food Res. 2021, 65, 2000712. https://doi.org/10.1002/mnfr.202000712

the epithelium by so-called enterocytes), the proteins may be further degraded by intracellular lysosomal proteases within the enterocytes.

The authors used a model to measure the potential capacity of these digested proteins to provoke allergic reactions. Four allergenic peanut proteins (Ara h 1, 2, 3, and 6) were incubated with pepsin at pH2.5 for 5 minutes, transported through intestinal tissue *in vitro* (using the InTESTine model), and subsequently measured for the indirect activation of basophils supplemented with IgE-antisera from peanut-allergic patients *in vitro*. Positive outcomes of this test would be a first indication of the potential capacity of the tested proteins/peptides to provoke a reaction in peanut allergy patients. Ara h 1 and Ara h 3 proved to be unstable towards pepsin, whilst both their digested and transported forms activated the basophils. By contrast, Ara h 2 and Ara h 6 were stable towards pepsin and only their transported forms were able to activate the basophils.

INFOGEST 2.0 digestion model

The INFOGEST 2.0 model[3] builds upon a previous model established through a European research effort. It is a static model which can be set up within a standard laboratory. It is aimed at increasing comparability of in-vitro digestibility research outcomes to the in-vivo system and to reflect the conditions of the upper digestive tract, particularly by including the oral phase. It entails the incubation of the test protein with subsequently:

- Amylase (simulated salivary fluid), pH 7
- Pepsin (simulated gastric juice), pH 3
- Pancreatin & bile (simulated intestinal juice), pH 7
- Addition of NaOH and a protease inhibitor AEBSF to stop the reaction, pH 7
- Methanol precipitation and collection of both the soluble fraction and pellet

Slight changes to the procedure (*e.g.,* lower pH of the gastric stage when studying probiotic survival) may be considered as well.

Discussion

EFSA explained that this agenda item was scheduled to introduce the aspect related to the protein safety assessment in GMO applications, showing limitations in certain cases, and in an urgent need for improvement. Czech Republic agreed that limiting the number of animal studies is needed. Czech Republic also asked whether in the future, information on the primary and secondary structure could be better used for the assessment of the proteins in GMOs. Germany acknowledged that the primary sequence does not provide enough information, and secondary and tertiary dimensional structure can be used since more experience and knowledge is becoming available. EFSA agreed that information on secondary/tertiary structure is actually generated and used for research purposes but not yet routinely used in risk assessment.
In relation to *in vitro* digestion studies, EFSA asked The Netherlands whether it will be possible to extrapolate information related to one species (i.e. human) to other animal species. The Netherlands acknowledged that some animal species can show

[3] Brodkorb, A., Egger, L., Alminger, M. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat. Protoc. 2019, 14, 991–1014. https://doi.org/10.1038/s41596-018-0119-1

allergic reactions, for example piglets to soybean. Moreover, in young terrestrial animals and fish, the barrier function of the intestine is not complete and proteins (e.g. growth hormones) might have a physiological impact. Very little attention has been given to some of these aspects.
Germany stated that some criticisms were raised on the CODEX Alimentarius consensus on the pepsin digestion test in relation to the pH level used. Moreover, testing combination of proteins could also take into consideration the protein expression levels. The Netherlands acknowledged that using different pH values in the digestion test could be an added value, but criticisms on the interpretability of the outcome have been raised. A more representative model of digestion is needed, by also taking into consideration the matrix effect. Germany mentioned that the focus should be on the weight of evidence approach and how to better choose the most appropriate experiments to be conducted. Slovenia commented on the history of safe use (HoSU) and on the criteria that should be used to prove it, also considering that exposure to certain proteins is difficult to be estimated. Slovenia also asked whether the criteria used in the Novel Foods area could be used to estimate the HoSU. Germany suggested that a distinction between toxicity and allergenicity to determine the HoSU. EFSA considered that reaching an internationally recognized definition of HoSU might be challenging but nevertheless some criteria could be identified. Italy stated that experience from other fields other than GMOs (e.g. novel food, insects, etc.) could be also considered. Hungary reported that applicants often refer to the HoSU to support the safety of the GMO in their applications. EFSA clarified that applicants' statements and the data provided in their support are always assessed by the GMO Panel and the outcome of such assessment is reported in the EFSA opinions. Germany acknowledged that although there is no agreed definition of HoSU, there are cases where the HoSU can be demonstrated and other cases where HoSU is not sufficiently demonstrated, and additional data may be needed. Ireland informed that HoSU concept is already in use in Novel Food area, and such definition has served the risk assessment reasonably well. EFSA welcomed more discussion and further contribution to the discussion on the protein safety issue from the Member States. The topic will be tabled again for future discussion.

Item 4: Visit to the Gene Bank facility

The Czech Republic host organized a guided tour to the Gene Bank facility at the Crop Research Institute. More information about the facility can be found **here.**

Item 6: Scientific opinion on new developments in biotechnology applied to microorganisms (LINK)

Abstract

EFSA presented an overview of the activities related to the mandate received on new developments in biotechnology applied to microorganisms. EFSA presented the results of the call for data open in the EFSA website from 7/03/2023 to 30/04/2023. EFSA reported that the response rate which included stakeholders from different areas, i.e., industry and academia was satisfactory. It was also pointed out that most of the stakeholders reported the use of CRISPR, and, in certain cases in combination with established genomic techniques. The products obtained fell into categories 3 and 4 as described in the EFSA Guidance (2011), of which, 61% accounted for category

4. The types of products included starter cultures, probiotics, biopesticides, biomasses, biostimulants, silage inocula, bioremediation products and other purified or non-purified products. Most of the products are expected to be on the market within the next 6-10 years according to the survey, while some of them have already been authorized elsewhere in the world.

EFSA also presented the ongoing work of the established working group to develop a scientific opinion on the topic. The working group, in accordance with the EFSA rules to address mandates, developed specific questions to address the three Terms of Reference received by the European Commission. These questions were presented during the meeting and feedback was requested. The working group had also identified some points (5 questions) which were shared in advance with the GMO Network and discussed during the meeting. EFSA staff committed to take back the comments to the working group.

New developments in biotechnology applied to microorganisms - Norway activity

Abstract

Norway (the Norwegian Scientific Committee for Food and Environment (VKM)) presented a summary from the VKM report "Genome editing in food and feed production – implications for risk assessment" (VKM 2021, link) of the findings regarding the applicability of the EFSA guidance for risk assessment of genetically modified microorganisms in risk assessment of genome-edited microorganisms. The report concludes: "*…the EFSA guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use is also applicable to genome-edited microorganisms. Due to the heterogenous uses of microorganisms/products their regulatory landscape can be considered complex, falling under both a directive, different EU regulations and various guidance documents developed by several of the EFSA panels. The product categorization presented in the guidance allows for differentiation in the amount of data needed for the assessment. In contrast to animals and plants, the core concept of qualified presumption of safety (QPS) provides a clear baseline for the comparative approach. This combined with a case-by-case approach provides both structure and flexibility to the risk assessment process. The same flexibility is offered to genome-edited organisms within this regulatory framework*."

Horizon scan and environmental risk assessment of GM virus applications

Abstract

Germany presented a summary on the horizon scan and environmental risk assessment of GM virus applications. The project run from Q4/2021 –Q4/2023 from the Environment Agency Austria and it was commissioned by BfN. The project assisted the horizon scanning exercises at CBD and OECD level and supported the implementation and enforcement of current GMO regulation. The project also supported further policy development. More information can be found in the presentation published at the EFSA website here.

Discussion

EFSA presented five questions to the Member States to stimulate the discussion on the new developments in biotechnology applied to microorganisms. The Netherlands asked whether the mandate included genome-edited microorganisms used in fields, for example biofertilizers. EFSA clarified that the mandate includes not only products in the FF area but also all the products which fall within the remit of EFSA, including for example biopesticides and biostimulants. EFSA clarified that different groups of microorganisms are included, like bacteria, yeast microalgae and viruses, and the contractor carrying the literature search is a consortium from Spain. Poland informed that lots of research is devoted to the field of endophytes. Regarding one of the proposed questions (i.e. 'If the same GMM can be obtained through different technologies how would the assessment be done?'), Poland also asked whether in case the same microorganism is obtained by different technologies, this would have an impact on the risk assessment, for example the use of different technologies would imply different requirements. EFSA replied that the working group experts are currently discussing this point and clarified that the mandate focuses on new developments in biotechnologies applied to microorganisms. Poland stated that the assessment should focus on the final product rather the technique used, and whole genome sequencing (WGS) should not be a problem for microorganisms to identify for example off-targets effect. Belgium asked clarification on the terms of reference, whether the mandate will look at the product or the technique. EFSA clarified that the terms of reference was interpreted as the assessment of the techniques. Belgium clarified that a hazard is a harmful characteristic of a GMO so applying this terminology to the technique as done in the terms of reference ('novel hazard of a technique') might be confusing. EFSA will bring this point up for discussion at the next working group meeting. Belgium referred also to the application currently under evaluation including a genetically modified microorganism (i.e. EFSA-GMO-NL-2019-162) which could serve as an example for this type of products. Belgium clarified that within Belgium the assessment of the application under the GMO Regulation focused on the molecular characterization part, as for the toxicity, allergenicity and nutrition aspects expertise was missing, and the guidance written by other EFSA panels seemed more fit for purpose to evaluate these aspects. An environmental risk assessment was not considered needed since no viable cells will be present in the final product and Directive 2001/18 is therefore not applicable. Regarding the need of the WGS, the Netherlands commented that the evaluation should be case-by-case and focus on the final application of the product, the mean of production and the exposure to the environment. Also, WGS may be requested as standard test, but the environmental risk assessment may require a case-by-case approach. Regarding the qualified presumption of safety (QPS) status and whether it should be extended to the GMMs developed through new genomic techniques, it was generally acknowledged that experience in this regard is still quite limited. The Netherlands stated that there could be cases where parental lines of the modified microorganism have no QPS status (e.g. growth of microalgae in confined environment and possible escape to the environment). Poland acknowledged that QPS is not a very familiar concept, whether it could be applied to known pre-assessed microorganisms with predefined genetic modification or more applied to new applications. EFSA clarified that the QPS is related to the microorganism, and it is a concept not related to the genetic modification *per se*. However, if the parental organism has QPS status, the status is extended to the genetic modification organism. In any case, the genetic

modification is assessed independently. Following this discussion, the Netherlands concluded that the extension of the QPS concept also to microorganisms modified by new genomic techniques should be possible.

DAY 2

Item 7: EFSA opinion on new developments in biotechnology applied to animals (including synthetic biology and new genomic techniques) (LINK)

Abstract

EFSA provided an overview of the mandate on New Developments in Biotechnology applied to animals. The status of the mandate, its progress and the deadlines were presented. The terms of reference were also presented. The drafting of a knowledge gathering report on known cases of animals and their food and feed products obtained by new developments in biotechnology is ongoing. It was reminded that this report will also be based on the outcome of a survey launched by a Contractor with expertise on the topic. The recruitment of experts is ongoing to setup an EFSA working group aimed to draft the scientific opinion on potential novel hazards/risks from new developments in biotechnology applied to current and near market animals and adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety & welfare, and environmental impact. With the progress of the mandate, EFSA will provide regular updates to the GMO Network.

New developments in biotechnology applied to animals – Norway activity

Abstract

Norway [the Norwegian Scientific Committee for Food and Environment (VKM)] presented a summary from the VKM-report (VKM 2021, link) of the findings regarding the applicability of the EFSA guidance for risk assessment of genetically modified (GM) animals in risk assessment of genome-edited (GE) animals. The VKM-report elaborates on a wide range of topics on health and environmental risk assessment of GM and GE animals with the use of five GE-animal examples.

The report concludes (not limited to animals): "*The inherent flexibility of the EFSA guidance makes it suitable to cover health and environmental risk assessments of a wide range of organisms with various traits and intended uses. Combined with the embedded case-by-case approach including the initial hazard identification step, that determines the type and extent of information needed for the assessment, the guidance is applicable to genome-edited organisms. VKM's evaluation has not identified new hazards specific to genome-edited organisms that fall outside the areas of concern established in the guidance.*

The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with novel traits (i.e., the phenotype of the organism) may be fully applied to all categories of genome-edited organisms.

The guidance on environmental risk assessment is largely concerned with novel traits and assessment of potential effects on biodiversity (e.g., in Norway) stemming from the spread and establishment of genome-edited organisms is fully applicable.

The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with the genetic modification (i.e., the genotype of the organism) may be fully applied to genome-edited organisms with inserted genes or long fragments of DNA, i.e., edits categorised as Site-Directed Nuclease type 3 (SDN3). However, these parts are not fully applicable for genome-edited organisms with minor insertions, deletions or single mutations, i.e., edits categorised as Site-Directed Nuclease type 1-2 (SDN1-2), edits obtained by oligonucleotide directed mutagenesis (ODM) or base editing (BE).

In summary, VKM finds that the EFSA guidance on risk assessment of genetically modified organisms provides a functional framework for risk assessment of genome-edited organisms. However, inclusion of specific considerations in the guidance regarding different properties of genome-edited organisms would be beneficial to ensure a common understanding between product developers and risk assessors regarding the type and extent of data needed to perform a risk assessment."

The VKM-report also states:

"Collectively, the guidance supplemented with technical notes covers new technological developments such as the potential use of omics and next generation sequencing technologies, as well as new genome-editing approaches. VKM emphasises that the overall relevance and suitability of the guidance is based on its dynamic nature. An assessment of the suitability of guidance should therefore not be limited to a narrow interpretation of the suitability of single documents."

Discussion

Austria asked Norway why certain parts of the EFSA guidance on the environmental risk assessment are applicable to SDN-3 applications but not on SDN-1 and SDN-2. Norway clarified that those parts of the guidance referring to the transgene insertion are not fully applicable, while EFSA guidances are overall applicable when the trait is the focus, e.g., in environmental risk assessment. EFSA asked Norway which part of the salmon sterile application have certain shortcomings. Norway clarified that there are several parts of the application that need improvement but could not provide further details at this time. Regarding the example on the virus resistant pig, Poland asked how the genetic modification (i.e., genetic deletion) is assessed if the same modification is already present in the natural gene pool. Poland stated that there could be cases when less amount of data would be needed for the risk assessment. Norway informed that there is an ongoing governmental project involving many stakeholders with the aim of discussing the regulation of biotech products in Norway. A draft report of this work was published on June 6th, the final report is expected in the autumn of 2023.

Belgium asked EFSA whether there are already applications on GM animal in the pipeline in the EU. EFSA clarified that the contractor has not finalized the search yet and the outcome will be provided when available. Czech Republic asked whether the contractor would search for information available outside EU and EFSA clarified that indeed the contractor is also searching for this type of information. Following a comment from The Netherlands, EFSA clarified that indeed the agency was involved in the assessment of DNA plasmid salmon vaccine CLYNAV in collaboration with EMA

(link) but EFSA was mainly involved in the assessment of DNA integration following an EC mandate. The Chair closed the discussion by suggesting the Member States to follow this mandate for further discussion in the new future.

Item 8: Request for placing on the market of Soy Leghemoglobin produced from genetically modified *Pichia pastoris* (EFSA-GMO-NL-2019-162) (LINK) – update

Abstract

AP162 is under risk assessment and the key issues in the evaluation were presented. Whilst the clock remains stopped for EURL GMFF deliverables, EFSA continues additional RA data requests as needed for these key issues. End of May additional data have been delivered and a further set of questions is likely to be sent to the applicant. The GMO Network was informed how the experts under the GMO Panel work together with experts under the FAF Panel, where a parallel evaluation takes place on the basis of a parallel dossier for the same product. The pending issues for MC, FF, and ERA (HGT) were explained and some detailed questions of the Member States on certain RA elements were addressed. As a future outlook, it was discussed how parallel evaluations can be avoided in the future by one leading Panel according to the use of the product.

Discussion

Austria asked clarification on the environmental risk assessment and how the evaluation is considering the presence of DNA in the final preparation with copies of resistance genes. EFSA clarified that there are neither resistance genes nor other genes of concern which remain in the final product. Belgium informed that discussion at the Member State level took place to decide the risk assessment strategy for this application. Belgium acknowledged the fact that the use of several guidances is needed for the risk assessment, and this is confirmed by the fact that EFSA is conducting the risk assessment by involving both the GMO and FAF Panel. Belgium asked EFSA whether the GMO Panel is needed for the evaluation of this product. EFSA also clarified that the European Commission was involved when discussing the applicable legal framework for this product. EFSA stated that there are procedures in place that would allow in the future only one Leading panel (i.e. the FAF Panel) to conduct the assessment for these types of products and additional expertise may be involved on a case-by-case. Belgium also asked about the additional information to be provided by European Union Reference Laboratory (EURL) and the related stop-the-clock. EFSA clarified that the risk assessment of the dossier would run in parallel while waiting the information to be provided to the EURL. EFSA also clarified that the EURL information related to the detection method is needed for the final adoption of the opinion according to the Reg. (EC) No.1829/2003. The Chair clarified that the safety of consumer and environment should be guaranteed with a fit-for-purpose assessment approach, for example with one single guidance for the RA of microorganisms. The Chair invited the Member States to further contribute to this discussion.

Item 10: Assessing safety-by-design in novel plant breeding techniques by comparing native gene-based modification with classical breeding

Abstract

Potential molecular effects of gene editing on crops have been studied in two recently finalized Dutch research projects sponsored by the national Ministry of Infrastructure and Water Management through the National Research Council's "Biotechnology and Safety" Research Programme. More information on the program can be found at the following website: https://www.nwo.nl/en/researchprogrammes/research-programme-biotechnology-and-safety

Some of the outcomes of research performed at Wageningen University and Research were also presented during the EFSA GMO Member State meeting on June 9th, 2023. In more detail, the projects were structured as follows:

Project #1 (led by Dr J Vossen): Assessing safety-by-design in novel plant breeding techniques by comparing native gene- based modification with classical breeding:

This project used conventional, gene-editing- and cisgenesis-based approaches for introducing late blight resistance genes into potato and their impact on the characteristics of the resulting potato lines. A safe-by-design strategy was followed by limiting the options for the selection of genes and methods of modification that could be used for this purpose. Statistical approaches were studied for their ability to support the interpretation of the wealth of data coming from extensive omics analyses performed on these crops. Moreover, the possibility to further refine the statistical approaches recommended by the EU guidelines for the safety assessment of GM crops has been explored (website: https://www.nwo.nl/en/projects/15815)

Further reading (examples of recent outputs):

- PhD Thesis: Monino Lopez, D. (2023) Breeding for potato late blight resistance in the era of precise genome editing. Wageningen University, https://edepot.wur.nl/589252
- Article: Kleter, G.A., Van der Voet, H., Engel, J., Van der Berg, J.P. (in press) Comparative safety assessment of genetically modified crops: focus on equivalence with reference varieties could contribute to more efficient and effective field trials. Transgenic Research, https://doi.org/10.1007/s11248-023-00344-y

Project #2 (led by Dr R. de Maagd): Specificity and side-effects of mutagenesis by nuclease-induced breaks and Cas9-mediated epigenome editing in plants; identifying hazards, analysing risks and creating inherent safety

This project investigated the precision and potential unintended effects of site-directed nucleases (CRISPR Cas-based) used for both gene editing in Arabidopsis thaliana *and tomato. These potential unintended effects include chromosome instability, chromosome deletions, and off-target mutations. Within its overview, it also took stock of such effects reported by others. The*

outcomes are considered to provide useful background information to the risk assessment community (website: https://www.nwo.nl/en/projects/15792)

Further reading (example of recent output):

- Slaman, E., Lammers, M., Angenent, G.C., De Maagd, R.A. (2023) High-throughput sgRNA testing reveals rules for Cas9 specificity and DNA repair in tomato cells. Frontiers Genome Editing https://doi.org/10.3389/fgeed.2023.1196763

Discussion

Czech Republic asked The Netherlands whether the insertion of resistance genes which disappeared from the breeders' gene pool could have an impact on the plant productivity. The Netherlands commented that reactivation of resistance genes might have implication also for the environmental risk assessment, for example if these genes render the crop a tolerant reservoir host for pathogens to other, susceptible plants. The Netherlands informed that there are overall 9 projects on biosafety and the outcomes of these projects will be published in the near future. The projects aim at assessing the impact of new future developmental technologies and would consider the safe-by-design approach. Also, The Netherlands informed that CRISPR-Cas is used to engineer fungal species for industrial production and the safe-by-design approach helps reducing off-target effects. Austria asked whether there is the need for decision criteria to be applied to assess whether the non-equivalence is biologically relevant. The Netherlands clarified that the statistical difference does not necessary indicate a risk and the experts' judgment is needed to interpret the non-equivalence scenarios and their relevance for the assessment. The Netherlands also added that the testing is more applicable to the food and feed area than to the environmental area. EFSA asked the Netherland whether any international reaction was received on the proposed approaches. The Netherland explained that the work was just presented at the OECD meeting and only recently published.

Item 11: Outcome of the EFSA's Scientific Colloquium 27 "Cell culture-derived foods and food ingredients" (LINK)

Abstract

Recent advances in fields such as tissue engineering, cell culture, and synthetic biology have paved the way for new technological approaches and products in the agri-food sector. Among these, cell culture-derived foods of animal or plant origin and food ingredients produced through precision fermentation are emerging.

In the EU system, such products require pre-market authorisation under different sectoral regulatory frameworks, such as the novel food and the food additives regulations, involving EFSA's scientific advice. Therefore, it is essential that EFSA's risk assessment methodologies and expertise in the field keep abreast of these technological developments.

The Scientific Colloquium brought together 80 in-person and 550 online relevant experts and stakeholders to discuss ongoing trends and research on the topic, and the requirements for keeping EFSA's risk assessment fit-for-purpose.

The Colloquium aimed to:

- Identify sectors in the agri-food system relevant to cell culture-derived foods and food ingredients;
- Review the state of the art of relevant concepts, technologies, and derived products;
- Discuss emerging safety and methodological aspects and their impact on EFSA's risk assessment approaches.

EFSA presented the outcome of the discussion of 2 breakout sections with potential relevance for the GMO Network (Break-out session 3 "New developments in engineered microbial cell factories: considerations for their safety assessment" and Break-out Session 4 "Development needs for the safety assessment of food ingredients derived from precision fermentation"). A detailed report including conclusions and recommendations for the future will be published in the coming months.

Discussion

The Chair clarified that the colloquium report will be ready in the following weeks. The Chair also informed that the scope of the colloquium was to improve awareness and preparedness for both EFSA and applicants since the regulatory framework to assess the products from precise fermentation is already in place. Germany asked whether the GMO network could be better informed about colloquia with potential relevance to GMOs. The Chair clarified that this colloquium was initiated by the Novel Food team within the NIF Unit. EFSA clarified that the communication of these initiatives may be also spread by the communication department in EFSA using distribution lists. EFSA will verify whether these distribution lists include the contact of the GMO Network. The Chair also suggests the participants to regularly look at the EFSA webpage to get informed about these types of events. Denmark noted that information on the colloquium was indeed provided by the Focal Point. Germany asked clarification on 2 applications on EFSA table to produce feed or feed additives from genetically modified organisms and asked whether the Member States can be informed when the applications will be valid and the modality of targeted consultation. The Chair clarified that feed additives applications will be assessed by the FEEDAP Panel. EFSA also clarified that upon validation the decision on Member States targeted consultation will be made. The Netherlands stated that not just the precision fermentation but also the discussion on cultured meat of the colloquium may be of interest to the GMO Network as this process may use genetically modified cells, recombinant proteins used as growth factors, etc. EFSA stated that an overview of the current discussion on cultured meat can be provided in the coming meetings to keep the GMO Network informed about this food area. The Chair informed that the colloquium's report will be ready during the summer and further discussion can be tabled at the next GMO Network meeting if needed.

Item 12: RNAi DEV project

Abstract

In order to keep guidance document for GM plants fit for receiving high quality dossiers, the scientific literature is regularly reviewed, and the risk assessment methodologies are checked against progress on the knowledge on the field. In January 2023, EFSA launched a literature search that will determine the need for an

update of the strategy for the risk assessment of GM plants developed using silencing approaches by RNA interference (RNAi).

Moreover, according to the Regulation (EU) No 503/2013, a bioinformatic analysis to identify potential 'off-target' genes is required for RNAi-based GM plants. In this context, EFSA is considering the development of a bioinformatic tool, to be made available to the applicants, to perform small RNA off-target bioinformatic studies offline in a secure environment. This tool will help automatise and harmonise analysis and data submission to help shortening risk assessment timelines.

Discussion

Czech Republic reminded that the assessment application of RNA as biocide (i.e. external application of RNA by spraying) is not under the GMO Panel remit and asked whether this is still the case. EFSA clarified that the GMO Panel participated in the past to the discussion on the assessment of RNA used a biocide, given the experience on bioinformatic in GMO applications. The situation has not changed since then, and RNA spray applications are not under the GMO Panel remit. EFSA clarified that the literature search will support the discussion on RNAi production within GMOs at the GMO Panel level. The GMO Network was encouraged to share information on RNAi activity performed at the MS level. EFSA will consider a way to collect such information if available. EFSA also clarified that the Member States comments received in the frame of targeted consultation on applications involving the use of RNAi has been taken into consideration.

Item 13: Any Other Business and communications

Belgium requested clarification about the new numbering system in place for the applications (i.e. EFSA question number, dossier number and EFSA internal sequential number) and noted that the communication on the dossiers is currently rather confusing as different numbers are used in different communications. EFSA provided a short presentation to address Belgium's requests for clarification, including an explanation of the application numbering system and OpenEFSA website search system. Germany also acknowledged that using different numbers may cause confusion. EFSA suggested that the same number could be used for consistency when communicating with the Member States, for example the dossier number (i.e. GMFF-20NN-NNN). Austria also informed that the search function in OpenEFSA does not always work well and sometimes retrieving the correct application is challenging. Austria also asked whether the numbering GMFF-20NN-NNN system would be also used in case of cultivation dossier since it may be specific for food and feed scope. EFSA will investigate this aspect and clarified that targeted and public consultations are triggered by the Salesforce system with the EFSA Risk Assessment Logistics (RAL) Unit involved in this process. EFSA clarified that in order to receive the notification on the public consultation, registration in Salesforce is needed. Moreover, it was clarified that the ESFC system automatically assigns the dossier number (i.e. GMFF-20NN-NNN) upon the e-submission of the dossier from the applicant, and EFSA assigns a question number (EFSA-Q-20NN-NNNNN) to handle the dossier. Belgium emphasized the need to be informed as soon as a targeted consultation is launched. EFSA suggested Belgium to contact their competent authority to verify who is entitled to receive the notification about the starting of the targeted consultation. EFSA also provided the contact point of RAL (i.e. RAL@efsa.europa.eu) to request help in

accessing the tools or in case of technical issues. Austria asked whether the limitation in number of characters in the targeted consultation has been extended and whether the upload function will be available. EFSA explained that the requested for change have been considered although the timeline for implementation is unknown. Belgium also asked whether there is a limit in the number of accesses to ESFC. EC will investigate the matter. Belgium also requested clarification on the presence of the watermarks in the documents and asked whether they can be removed in the confidential dossier to ease the reading by experts. EFSA clarified that watermarks have been reduced by the applicants, but they are required by regulation for the submission phase. Nevertheless, EFSA suggested the Member States to address all these technical questions to RAL. EFSA also suggested that, in cooperation with RAL, some clarifications could be sent to the Member States representatives involved in the targeted consultation.

Closure of the meeting

The Chair thanked the GMO Network members 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 13:00.