Network on Risk Assessment of GMOs
Minutes of the 7th meeting

Held on 31 May – 01 June 2016, Parma
(Agreed on 22 March 2017)

Participants

- **Network Representatives of Member States (including EFTA Countries):**

Country	Name
Austria	Dietmar Vybiral, Markus Woegerbauer
Belgium	Rene Custers, Katia Pauwels
Bulgaria	Dimitar Djilianov, Tzveta Georgieva
Cyprus	Andri Varnava Tello
Croatia	Hrvoje Fulgosi, Sanja Milos
Czech Republic	Miloslava Navratilová, Vladimir Ostry
Denmark	Jan Pedersen
Estonia	Andres Mäe
Finland	Kirsi Törmäkangas, Annikki Welling
France	Catherine Golstein, Emmanuelle Pic
Germany	Wolfram Reichenbecher, Andrea Scheepers
Greece	Margarita Karavangeli
Hungary	Barnabás Jenes, Ágnes Fejes
Ireland	Thomas McLoughlin, Patrick O'Mahony
Italy	Roberta Onori, Elena Sturchio
Lithuania	Zygimantas Janeliunas, Odeta Pivoriene
Netherlands	Boet Glandorf
Poland	Zbigniew Dąbrowski, Slawomir Sowa
Slovakia	Petra Gerekova
Slovenia	Martin Batic, Boštjan Petelinc
Spain	Carmen Cuadrado, Gema Perez
Sweden	Christer Andersson
Norway	Merethe Aasmo Finne, Ville Erling Sipinen
Switzerland	Martin Schrott

- **European Commission:**

Panagiotis Daskaleros (DG SANTE).

- **EFSA:**

GMO Unit: Fernando Alvarez, Herman Broll, Fabrizio Chiaramonte, Yann Devos, Antonio Fernández Dumont, Andrea Gennaro, Anna Lanzoni, Claudia Paoletti,

Konstantinos Paraskevopoulos, Matthew Ramon, Regina Selb and Elisabeth Waigmann (Chair).

SCER Unit: Reinhilde Schoonjans (for agenda point 6.1).

GMO Panel Members: Josep Casacuberta, Antoine Messéan and Hanspeter Naegeli (Chair of the GMO Panel).

Members of GMO Panel Working Groups (WGs): Simon Moxon (MC WG) and Joe Perry (ERA WG).

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Ms Louise Ball (UK) and Ms Esther Kok (Netherlands).

2. Adoption of agenda

The agenda was adopted without changes.

3. Agreement of the minutes of the 6th meeting of the Network on the Risk Assessment of GMOs held on 12-13 May 2015, Parma

The minutes were agreed by written procedure on 30 November 2015 and published on the EFSA website 09 December 2015.

4. Topics for discussion

4.1 Update on recent and current EFSA activities on GMOs – mandates, guidance documents, procurement and grants

Elisabeth Waigmann, Head of the GMO Unit, presented the topics discussed at the 7th meeting of the GMO Network and indicated for which of them EFSA or the GMO Unit, in particular, had follow-up actions. She continued by offering an overview of the recent and current EFSA activities on GMOs, including applications, guidance documents, external mandates, and procurement and grants.

There were no questions asked on this presentation.

4.2 Guidelines on possible derogation to existing requirements for applications of genetically modified (GM) food and feed at low levels submitted under Regulation (EC) No 1829/2003 on GM food and feed

Emmanuelle Pic, delegate from France to the GMO Network (MC/FF), gave a talk on the possible derogation to existing requirements for applications of genetically modified (GM) food and feed at low levels submitted under Regulation (EC) No 1829/2003. Her talk was based on a scientific and technical support note prepared by the 'Biotechnology' working group at ANSES at the request of the French competent authority following the European Commission mandate to EFSA to develop a guidance document on this topic. The scope of the exercise was restricted to genetically modified (GM) plants and to the

assessment of the health risk for humans and animals, and covered both raw and processed products. Two separate approaches are described depending on whether the products are consumed as a whole or only in food/feed after mixture and/or processing. The appraisal took into account thresholds of 0.9%, 0.5% and 0.1% for the presence of GM material. However the proposed derogation to existing requirements for applications of GM food and feed at low levels was not different between the three proposed thresholds.

For products consumed only in mixtures and/or after processing, it was suggested that there should be no derogation from (i) a complete molecular characterisation; (ii) a 28-day toxicity study if the newly-expressed protein(s) (NEP) has (have) never undergone a toxicological assessment; and (iii) the study of possible allergenicity changes linked to the genetic modification(s) for plant species known to be highly allergenic.

For food products that can be consumed as a whole, (i) and (ii) were considered necessary as above. For (iii), in addition to the above, an allergenicity assessment of the NEP according to the EFSA recommendations was also considered necessary if the concentration of the NEP(s) in the edible tissues reaches a magnitude of 1 milligram per gram of dry weight. In addition, a complete compositional analysis should be provided, based on the parameters described in the OECD consensus documents and performed in accordance with the EFSA recommendations, if the trait(s) introduced in the GM plant is (are) designed to change its composition or certain metabolic pathways. In other cases, this analysis may be limited to anti-nutritional, toxic and allergenic compounds.

The appraisal also stresses the fact that applicants should be requested to also provide the reference material and the method for detecting and quantifying the presence of the GM plant under assessment, as necessary for standard applications for marketing authorisation under Regulation (EC) No 1829/2003.

The presentation was followed by a general discussion.

A delegate from Poland commented that the molecular characterisation would need to be similar to that of regular GM applications submitted under Regulation (EC) No 1829/2003, and that quantification methods should be developed. Ms Pic replied that the quantification methods would be submitted to JRC for evaluation, together with reference material, as for full applications. She agreed that molecular data requirements should be the same as for regular GM applications.

A delegate from Austria wished to clarify that there would be a difference between applications for authorisation of events to be marketed in the EU, and applications for events that might be present at low levels (Low Level Presence or LLP) in shipments to the EU; the latter would have less data requirements for risk assessment. The applicant would be in the position to choose whether to apply for full import/processing or just for LLP, for which the 0.9% threshold would apply. Ms Pic noted that the 0.9% threshold for labelling is applicable to authorised events and that currently there is a zero tolerance for unauthorised events on the EU market. She clarified that, if an event has an authorisation only

for LLP but is present at levels higher than 0.9%, it would be treated as an unauthorised event.

A delegate from Finland asked how the 0.9% threshold would be applied to whole fruits, giving the example of the GM papaya. Ms Pic replied that in this case sampling will be very important, as it would need to ensure that enough fruits are tested from a certain lot. She noted that the general approach for products consumed as a whole would need to take into account that the consumer might pick the GM fruit from a lot, therefore being exposed to 100% of the GM event in question. It would also need to take into account the repeated exposure of the consumer, since this situation could happen again, according to the composition of the successive lots that arrive in the store.

A delegate from Switzerland asked how the Codex Alimentarius guidelines on LLP were taken into account (Codex Alimentarius, 2009a). Ms Pic replied that other guidelines on this topic, such as Codex Alimentarius and OECD guidelines, have limited scope; for food only (not feed), and for environmental risk assessment of seeds or grain commodities respectively.

A delegate from the Netherlands enquired whether information submitted by applicants as part of the authorisation process in other countries can be used when submitting for LLP application in the EU. Ms Pic replied that these data can be used if they correspond to the derogations that will be described in the future guidelines and that the assessment will be done on a case-by-case basis.

EFSA asked for further details on how the allergenicity assessment would be performed. Ms Pic replied that the allergenicity assessment will be carried out according to the EFSA recommendations if the concentration of the newly expressed protein(s) in the edible plant parts is higher than 1 mg/g dry weight; furthermore, an assessment on the possible changes in allergenicity associated with the genetic modification will be required for plants known for their natural allergenicity (e.g. soya, fruit with allergenic properties, such as kiwi and papaya).

Anna Lanzoni, a senior scientific officer of the GMO Unit presented the current status of activities on the European Commission (EC) mandate to EFSA to develop guidance on possible derogation of existing requirements for applications of GM foods and feeds at low levels under Regulation (EC) No 1829/2003. More specifically, the background information and the terms of reference of the mandate were described, as well as the planned next steps including the involvement of stakeholders.

In 2009, Codex Alimentarius developed specific guidelines for the food safety assessment of LLP situations of GM material to be applied in pre-market risk assessment of LLP applications. It was also reiterated that LLP applications should only concern GM products not intended for the EU market and therefore not for GM products for which a full scope application was previously submitted.

Based on the EC mandate, EFSA is requested to advise whether or not the requirements of Annex II of the Implementing Regulation (EU) No 503/2013 are necessary to conclude on the safety of GM events in applications, covering the

unintended presence of GM products in food and feed at the adventitious or technically unavoidable presence threshold of 0.9% or below, and if there is the possibility for derogations from Annex II - in this case a rationale should also be given. The mandate was accepted in July 2015 and an *ad hoc* 'LLP Working Group' was set up, consisting of experts from the MC, FF and ERA standing WGs of the GMO Panel, as well as members of the EFSA GMO Unit. The first draft of this guidance document is foreseen for endorsement by the GMO Panel and a subsequent dedicated EU Member State consultation in autumn of 2016. A second consultation, open to the public (including EU Member States), is foreseen in spring 2017. Finalisation of this guidance is foreseen in September 2017.

The presentation was followed by a general discussion.

A delegate from the Netherlands asked how the environmental risk assessment will take into account spillage. A GMO Panel member replied that the WG is considering using a decision tree for the environmental risk assessment of LLP events, focusing on the type of crop and trait of the respective GMO. He noted that for exotic species or new traits it might be difficult to apply such a decision tree, so discussion is still on-going in the LLP WG on this topic.

Delegates from Denmark and Poland asked whether the LLP guidance under development by EFSA covers also the scenario of an LLP GM soybean event present, for example, in a maize shipment. EFSA clarified that this issue had been discussed with the European Commission and the outcome was that this situation would not be within the scope of the LLP guidance; what the LLP guidance covers is the low level presence of a GM ingredient into an ingredient of the same kind.

A delegate from Belgium asked whether the information on the detection and quantification method for the LLP GM event should be submitted as part of the LLP application, to which EC replied positively. A representative from Sweden asked whether the LLP guidance will be applicable only to GMOs to be used for food and feed purposes, or also to those developed for technical purposes (e.g. biofuels), to which EFSA replied that it would be only for GMOs used for food and feed.

A delegate from Switzerland asked whether scenarios of repeated exposure and presence of several LLP GM events in a food product were taken into account. EFSA confirmed that "technically unavoidable" does not exclude repeated exposure, especially in the case of GM events consumed as mixtures (e.g. cornflakes). As for the presence of several LLP GM events, EFSA clarified that although each LLP application will be a standalone application covering one single vent or stacked events, a cumulative assessment should be done for events with similar traits. To support this, compositional data on the relevant compounds should be provided.

A delegate from Poland mentioned that sampling to ensure the detection and quantification of LLP GM events, in particular for GM fruits, might be challenging.

A delegate from Belgium asked whether the LLP guidance would be applicable also to plants developed through new breeding techniques, to which EFSA replied that this is a decision for risk managers, namely the EC.

4.3 Break-out session MC/FF: Allergenicity guidance development; current status and next steps

Antonio Fernandez-Dumont, the scientific officer of the GMO Unit in charge of the allergenicity guidance development presented the most recent activities on the EFSA's self-task developing supplementary allergenicity guidelines for the risk assessment of GM plants. Information was provided on the main aspects to be discussed in the draft document, which focuses on i) non-IgE-mediated immune adverse reactions to foods, ii) *in vitro* protein digestibility testing, and iii) endogenous allergenicity. It was also explained that the aim of this guidance document is to provide supplementary guidelines and not to challenge the main principles of the allergenicity assessment of GM plants. The motivation for this self-task was the need to: i) consider new developments in the area; ii) address recurrent comments and questions from stakeholders; and iii) assist applicants and risk assessors in the practical implementation of the requirements laid down in the Implementing Regulation (EU) No 503/2013. Finally, the procedural steps in the process until adoption (foreseen in the spring of 2017) were explained.

The presentation was followed by a general discussion.

A delegate from Denmark asked how EFSA, using this proposed new guidance document, will risk assess differences in the levels of endogenous allergens between the GM plant and its conventional counterpart, and whether there is substantial variation in the levels of endogenous allergens in conventionally bred plants; if so, how would this be taken into account. EFSA clarified that in Codex Alimentarius (2003; 2009b) endogenous allergenicity is a relevant aspect to be considered. Endogenous allergens are very relevant also in traditional crops, and allergic individuals know from experience the amount of offending food that they can tolerate. EFSA also acknowledged that from a scientific point of view, unintended effects may be considered for all crops and not just for GMOs. However, the current principles for the safety assessment of GMOs rely on the comparative assessment where a GM plant is compared with its appropriate non-GM comparator and where natural variability is further considered for the overall assessment. The Implementing Regulation (EU) No 503/2013 requests the measurement of relevant individual allergens on crops recognised to be allergenic. The guidance document under progress will mainly focus on developing a strategy to comply with Implementing Regulation (EU) No 503/2013 for endogenous allergenicity, and will consider soybean as an example.

A delegate from France asked which tests might be replaced by the suggested *in vitro* digestibility test and whether this suggested method might undergo a testing phase before it is implemented. EFSA clarified that the objective is not to replace the existing pepsin test but probably to complement or modify it, taking into account the latest scientific developments. EFSA also clarified that there might be indeed a need for a testing phase of the 'optimised' *in vitro* digestibility test before any requirement is implemented.

A delegate from Germany asked how EFSA would implement such data in a comparative approach in cases where a relevant difference is found and how would the results be interpreted considering that there would be no threshold in place. EFSA replied that the equivalence test already in place as part of the comparative approach will be used. EFSA also mentioned that the upcoming public consultation is an opportunity to receive useful feedback on this issue.

A delegate from Sweden asked whether the proposed methods would be applicable for both atopic and non-atopic patients. EFSA responded that the guidelines should in principle be applicable for sensitisation and elicitation. Currently, data showing an association between allergen dosage and allergic sensitisation are scarce. However, there is substantial experimental evidence suggesting a population dose-distribution relationship for elicitation.

4.4 Break-out session MC/FF: Role of sampling in the risk assessment of GM Plants

Claudia Paoletti, deputy Head of the GMO Unit, presented the background and the recent activities linked to the topic of sampling of plants in the context of studies typically provided for GM crop risk assessment. The focus was on: (i) the importance of using correct sampling procedures when collecting data for FF risk assessment; (ii) the need of representative sampling to correctly take into account material-specific heterogeneity; (iii) the existing "Theory of Sampling" as a framework for representative sampling; and (iv) the on-going EFSA service contract.

The presentation was followed by a general discussion.

A delegate from Denmark made a number of comments focusing on the applicability of sampling strategies given that sampling is normally considered primarily for GM contamination reasons. He pointed out that compound levels in a plant may differ significantly from one generation to another and therefore plants that are in the market might not be represented by what was originally assessed in the risk assessment stage. EFSA replied that sufficient information from the risk assessment stage should be obtained and this information should be as accurate as possible. A member of the GMO Panel commented on the need for representative sampling by explaining the importance of correct sampling for comparative assessment and for determining the levels of the newly expressed proteins, given that only samples (and not all plants) are analysed.

A delegate from Sweden mentioned that the importance of sampling in the RA of GM crops is acknowledged and that ensuring the most representative sampling should be a general aim for all products and not only for GM crops. EFSA agreed that ensuring correct sampling is a horizontal issue and informed the participants that the outcome of the GMO Panel activity would serve as a pilot study for other units of EFSA.

A delegate from France asked whether the final objective of this work is to provide guidance on how the applicant should perform sampling. EFSA replied that the output of this work would be a contractor report and not a guidance document. It should however encourage applicants to better document their sampling methods. The delegate from France also asked how these methods

would be extrapolated from one field to the next. EFSA clarified that this point lies at the core of the issue and discussions are currently on going to evaluate whether or not the distribution pattern of variability of an endpoint can be regarded as constant regardless of the location of growth of the plant.

A delegate from Poland asked if sampling is considered a process for testing/analysis or rather a method to obtain representative data; if the latter is the case then there should not be any difference between sampling for GMOs and any other material since it is a statistical approach. He also commented that it is a matter of how much uncertainty would be considered acceptable. If a limit on the acceptable uncertainty can be set then sampling will be considered the same regardless of whether we are dealing with GMOs or non GMOs. EFSA replied that representative sampling would indeed be applicable regardless of the type of samples. He also commented that a threshold should be set to define how precise the information should be considering that in many cases, a range of variation of the population would be sufficient with no need for high precision. EFSA replied that in a number of cases, it might be difficult to define a threshold for risk assessment but having reliable sampling information would improve confidence in the submitted data. The delegate from Poland also asked whether the need for guidance on sampling is a consequence of insufficient information provided by applicants in current GMO applications (e.g. number of plants analysed). EFSA clarified that the amount and quality of provided information has been variable and the ongoing activity aims at better defining the sampling procedure thereby harmonising the data needs.

A delegate from Italy commented that correct sampling is a horizontal issue and it is important for the risk assessment of GMOs to keep the errors as low as possible.

A delegate from Switzerland asked whether variation within a field can be estimated for sampling purposes in terms of number of years, sites etc, considering that field testing might need several years to capture variation. EFSA replied that variation can be triggered by several factors (environment, agricultural practises etc). Sampling is the tool to estimate such variation so if sampling errors can be effectively corrected, actual variability can be estimated more accurately.

4.5 Break-out session ERA: EFSA's recommendations on resistance monitoring for corn borers

Fernando Alvarez, scientific officer of the GMO Unit, presented EFSA's recommendations on resistance monitoring for *Ostrinia nubilalis* (European corn borer, ECB) and *Sesamia nonagrioides* (Mediterranean corn borer, MCB) (EFSA, 2015; EFSA GMO Panel, 2016). Based on the outcome of model simulations for ECB populations to estimate the number of generations required to evolve field resistance to the Cry1Ab protein expressed in maize MON810 (i.e., resistance allele frequency of 50%), and considering the time needed to implement appropriate risk mitigation measures, the GMO Panel advocates setting the minimum detection limit for resistance allele frequency at 3%. To achieve this detection limit, at least 1,000 ECB and MCB larvae should be collected in field for testing purposes. Since field resistance to Cry1Ab is more likely to evolve in

areas where the adoption rate of maize MON810 is high, sampling efforts should focus on the Ebro valley, where adoption rates of maize MON810 have been the highest in the Iberian Peninsula since 2003. It is recommended to collect samples in three zones of approximately 10 km × 10 km, where the adoption rate of maize MON810 is higher than 50% for at least three consecutive years. The consent holder should clearly identify cases where larger zones are required to ensure that sufficient numbers of larvae are collected. Both target pests should be sampled annually, because corn borer populations can complete two generations per year in north Spain. The GMO Panel also recommended the consent holder to provide further details on the sampling and testing methodology in the future annual PMEM reports on maize MON810.

The presentation was followed by a general discussion.

A delegate from the Netherlands noted that a public GMO register could help to inform where sampling should be conducted. A delegate from Spain clarified that such a register is not in place in Spain. The lack of exact data on maize MON810 cultivation and adoption at the field level in the Ebro valley hampers the possibility to determine with certainty where the adoption rate will be the highest *ex ante*. Cropping history data give an indication on the average uptake over time. Currently, the consent holder relies on sales data of seed to get an indication of the province where maize MON810 may be grown. A member of the GMO Panel emphasised it would be advisable to improve the current GMO register system in Spain, but that the final decision on this matter is a risk managerial issue that is not in the remit of EFSA.

A delegate from Poland asked if Spanish farmers comply with the refuge requirements and carry out the operational details of insect resistance management plans. He also seeked clarifications on whether attempts have been made to collect larvae in maize MON810 fields. EFSA indicated that more than 90% of the farmers comply with the refuge requirements in Spain. EFSA reminded that the high-dose/refuge strategy prescribes planting *Bt*-crops that produce a very high concentration of the *Bt*-toxin (25 times the amount needed to kill [99 % of susceptible individuals [LC_{99}]), so that nearly all target insect pests that are heterozygous for resistance do not survive on it. In addition, a nearby structured refuge of the non-*Bt*-crop is required where the target insect pest does not encounter the *Bt*-toxin. When cultivating maize MON810, the presence of refuge areas equivalent to at least 20% of the surface planted with maize MON810, should be ensured when a single field cropped to maize MON810 is larger than 5 ha, and when a cluster of adjacent fields cropped to maize MON810 has an aggregated surface greater than 5 ha, irrespective of individual field and farm size. EFSA also clarified that sampling is mainly performed in non-*Bt*-maize fields (including refuges), as most susceptible larvae are killed in maize MON810 fields.

A delegate from Belgium commented that resistance allele frequencies above a detection limit of 0.5% have not been reported after ten years of maize MON810 cultivation. EFSA replied that model predictions do not expect resistance to evolve before 20 years, provided that farmers comply with the refuge requirements and carry out the operational details of insect resistance management plans. Nonetheless, the potential for corn borers to evolve resistance to Cry1Ab has been identified as a risk by the GMO Panel. The GMO Panel therefore advised that appropriate insect resistance management

strategies continue to be employed, in order to delay and monitor resistance evolution.

A delegate from Finland wondered whether the number of larvae to sample is proportionate to the detection level to reach. A member of the GMO Panel explained how the confidence interval is calculated, and clarified that 1,000 larvae are needed to ensure that the allele frequency remains below the 3% in case no resistant larvae are detected.

4.6 Break-out session ERA: Potential exposure of NT lepidopteran larvae to Bt-maize pollen deposited on their host plants

Joe Perry, member of the GMO Panel's ERA WG, presented the GMO Panel scientific opinion updating risk management recommendations to limit exposure of non-target (NT) Lepidoptera of conservation concern in protected habitats to *Bt*-maize pollen (EFSA GMO Panel, 2015). Using mathematical modelling, the GMO Panel had previously quantified (e.g. EFSA, 2012) the risk to NT Lepidoptera of conservation concern, potentially occurring within protected habitats, associated with the ingestion of *Bt*-maize pollen deposited on their host plants. To reduce the estimated larval mortality to a negligible level, an isolation distance of 20m and 30m was recommended between protected habitats and the nearest fields of maize MON810/Bt11 and 1507, respectively. In EFSA GMO Panel (2015), the GMO Panel refined its model predictions, accounting for newly reported information on maize pollen deposition over long distances. An analysis of various sources of uncertainties affecting the exposure of NT Lepidoptera to *Bt*-maize pollen was conducted, in order to provide quantitative estimates of realistic exposure levels. The GMO Panel concluded that its previous recommendation for a 20m isolation distance around protected habitats, within which maize MON810/Bt11 should not be cultivated, remains valid. New calculations show that the previously recommended isolation distance of 30m from the nearest maize 1507 field would still protect NT Lepidoptera with known levels of sensitivity, including the 'highly-sensitive' *Plutella xylostella*. Should hypothetical species with greater sensitivities exist, larger isolation distances would be needed to ensure the desired level of protection.

ERA conclusions and risk management recommendations previously made by the GMO Panel were also discussed in light of new scientific publications by Hofmann et al. (2014, 2016) and Lang et al. (2015). It was explained that EFSA will receive a mandate from the European Commission to assess whether these new scientific publications contain elements that would lead the GMO Panel to reconsider the outcome of its previous scientific opinions.

The presentation was followed by a general discussion.

A delegate from Croatia asked whether meteorological conditions and other factors affecting Cry1Ab degradation are taken into account in the model. Mr Perry replied that these aspects are considered, though some of these aspects are subject to uncertainty, as described in EFSA (2015). Real time data, such as those gathered in the frame of the AMIGA project, could help to fine-tune the model predictions.

A delegate from the Netherlands asked why target species are not considered the most sensitive ones since they contain the specific receptors for the Cry

protein, and therefore more sensitive to Cry1Ab than other non-related species such as endangered/protected species. EFSA clarified that the level of sensitivity between-species is not necessarily correlated with their pest or protection status. For example, EFSA Scientific Committee (2016a) indicated that there is no conclusive evidence that endangered/protected species are *per se* more sensitive towards potential stressors than other species. Mr Perry added that the number of lepidopteran species tested to date is low, and therefore uncertainty remains about the sensitivity distribution of species. The GMO Panel therefore decided to follow a conservative approach by considering a range of categories of species sensitivity, including hypothetical categories for which no actual species have yet been recorded with that degree of sensitivity.

A delegate from France asked whether the distribution of sensitive NT species beyond exposed margins should be taken into account in the risk assessment associated with exposure of NT lepidoptera larvae to Bt-maize pollen deposited on their host plants, in order to assess the relative impact of the Bt crop on a given population. Mr Perry replied that distribution should be considered and current EFSA risk assessments do take this into account. However, little is known about the sensitivity of most NTO lep larvae and the only species for which bioassays have been performed to estimate sensitivity to Bt toxin are Inachis io, Vanessa atalanta and Vanessa cardui. In addition, some information is known of about 15 lep species for maize 1507 pollen and can be used to gain an insight on the likely frequency distribution of sensitivities. EFSA have extrapolated using these data to other hypothetical species for their risk assessments in a conservative fashion to account for the uncertainty.

Delegates from Germany and the Netherlands did not consider the on-going scientific debate via rebuttals and other written responses between EFSA and several authors of publications criticising EFSA's model the most efficient and constructive way of exchange. These delegates favoured a more open dialogue between the involved parties. EFSA mentioned that practical constraints (e.g., tight deadlines imposed by the mandate requestor) render it challenging to engage in an open dialogue in all cases, but took note of the suggestion. Mr Perry added that EFSA has the legal obligation to take into account new information relevant for the risk assessment of GMOs, and consider it formally. Within this context, a delegate from France asked whether EFSA approached the authors of the critical publications to request raw (non-published) data. Mr Perry replied that such data were requested, but not provided by the approached authors.

4.7 Scientific Committee guidance on specific protection goals for use in ERA

Reinhilde Schoonjans, scientific officer of the Scientific Committee and Emerging Risks (SCER) Unit, presented the three latest outputs on environmental risk assessment (ERA) adopted by the Scientific Committee (SC):

- Scientific opinion on coverage of endangered species in ERA (EFSA Scientific Committee, 2016a);
- Scientific opinion on recovery in ERA (EFSA Scientific Committee, 2016b);

- Guidance to develop specific protection goals options for ERA, in relation to biodiversity and ecosystem services (EFSA Scientific Committee, 2016c).

At EFSA's 10th anniversary conference[1], it became apparent that EFSA's ERA schemes have evolved independently in the different areas within its remit, and that further harmonisation might be possible on specific topics. EFSA therefore mandated[2] the SC to harmonise EFSA's ERA schemes with regard to: (1) developing options for specific protection goals (SPGs) for ERA in relation to biodiversity and ecosystem services; (2) coverage of endangered species in EFSA's ERAs; and (3) temporal and spatial recovery of non-target organisms for ERAs.

The Guidance presents a science-based framework to make general protection goals operational for use in all areas of EFSA's ERAs by accounting for the importance of ecosystems and biodiversity in providing benefits to humans. The ecosystem services approach proposed by EFSA follows three sequential steps: (1) the identification of relevant ecosystem services; (2) the identification of service providing units for these ecosystem services; and (3) the specification of options for the level/parameters of protection of the service providing units using five interrelated dimensions. This last step involves the specification of options for the ecological entity and attribute to protect and the magnitude, temporal scale and spatial scale of the biologically relevant and, in the case of regulated products, tolerable effects.

The ecosystem services approach provides an easy-to-understand tool and a common language, which facilitates communication among stakeholders. Improved communication will help to clarify the often divergent positions on what is of value and why, and reveal the underlying values and ideals held by the different actors. Communication among stakeholders will also be essential to reach agreement on operational protection goals, which must be set before risk assessments are conducted, as they define the framework in which scientists and risk assessors operate when performing the risk assessments.

Setting the level of protection necessitates a dialogue between risk assessors and risk managers, because it involves normative considerations, which cannot be accounted for by risk assessors and scientists alone. Means to facilitate this dialogue were discussed.

The presentation was followed by a general discussion.

A delegate from Belgium wondered whether the approach proposed to make protection goals operational is hypothesis-driven, and hence stressor-specific. EFSA replied that the early steps of the ERA (i.e., problem formulation) establishes the context for the risk assessment by identifying which of the potentially exposed and susceptible components of the environment (species, habitats, services, etc.) are valued by civil society and/or protected by relevant laws or policies. Ideally, protection goals should be identified independently from the stressor. It was acknowledged that putting the ecosystem services concept into practice can entail challenges due to the complexities of ecosystem

[1] EFSA (European Food Safety Authority), 2012. EFSA@10 Conference proceedings – challenging boundaries in risk assessment – sharing experiences. Available online: http://www.efsa.europa.eu/it/events/event/121107

[2] Mandate M-2013-0098 in the EFSA Register of Questions

components and their interactions, and lack of understanding of how regulated products may impact ecosystem service delivery across different spatial scales. Yet, several delegates agreed that what constitutes environmental harm should be defined more clearly and precisely, independently from the regulated product under consideration. They concurred that this would facilitate a more structured approach to ERA, ensure a common approach on how to derive operational protection goals across different regulated products under EFSA's remit, and increase the value of ERAs by providing information necessary for effective regulatory decision-making (Devos et al., 2015, 2016).

Mr Perry asked whether the EC should set protection goals, and to which extent EU Member States are involved in this process. He noted that there could be substantial differences on the level of protection to achieve between EU Member States and between the EC and EU Member States. EFSA indicated that EC standards are used for its ERAs, and that EU Member States can fine-tune EFSA's ERAs to account for national/regional and local specificities. A delegate from Finland added that there are situations where protection goals are defined by EU Member States. The need to improve the communication among the EC and EU Member States was considered essential to reach agreement on operational protection goals.

A delegate from Sweden requested clarifications on the definitions used to define and categorise endangered species. EFSA replied that endangered species are defined as a species that is either: (1) listed in one or more "red lists" as threatened (i.e. vulnerable, endangered, or critically endangered, or variants thereof), where the considered red lists are: (i) the European Red List, (ii) the global IUCN Red List of Threatened Species, and (iii) national and other regional red lists within Europe that follow the IUCN or another suitable classification scheme; or (2) rare based on Rabinowitz's seven classes (Rabinowitz, 1981) of rarity (including "endemics", "classic rarity", "habitat specialists" and "truly sparse" species) (EFSA Scientific Committee, 2016c).

4.8 Next generation sequencing in the risk assessment of GMOs

Simon Moxon, member of the GMO Panel's MC WG, gave a talk on Next Generations Sequencing (NGS) technologies, applications and potential issues. After a historical overview on the development of the NGS technology, the potential of this technology as a powerful, cost-effective tool was presented as well as its widespread applicability in several areas such as DNA analysis (e.g. genome/exome sequencing and DNA methylation), RNA analysis (e.g. RNAseq and miRNA identification) and protein analysis (e.g. protein-DNA and protein-RNA interactions). Mr Moxon also presented a typical NGS workflow, from experimental design, sample collection and library preparation to the sequencing steps and the subsequent data analysis using bioinformatics tools. Several critical factors such as number of replicates, sequencing depth and read length were also discussed. In addition, current limitations with storage and computing power requirements as well as the non-homogeneity among a vast number of bioinformatics tools were pointed out. Moreover, a suggested set of minimum data requirements for the use of NGS methods in the context of the molecular characterisation data in GM applications was presented; this set of data includes number of reads, minimum sequencing length, quality control results, inclusion

of all in-house developed code etc. Finally, given the complexity and variability in the approaches of the NGS methods, the need to have a standardised methodology that would allow risk assessors to evaluate NGS-derived data in a consistent way was discussed. As NGS could become a standard method to characterise GMOs in the near future, there might be a need to work towards a set of guidelines to set minimum requirements.

Josep Casacuberta, member of the GMO Panel and Chair of the MC WG, gave a talk on the use of NGS techniques for the molecular characterisation (MC) of GM plants in the frame of the Implementing Regulation (EU) No 503/2013 and the relevant EFSA guidance document (EFSA GMO Panel, 2011). As laid down in these documents, GM plants need to be characterised in terms of i) sequences inserted; ii) potential disruption of known coding and regulatory sequences; iii) analysis of the integrity/stability of the insert(s); and iv) analysis of the expression of the inserted sequences.

With regards to point i), the structure of the insert is typically analysed by PCR and Sanger sequencing, and copy number and analysis of the potential presence of backbone/vector sequences are normally characterised by Southern analysis. NGS could substitute these methods in defining the insert and the number of junctions thereby indicating the number of insertions. With regards to point ii), analysis of the insertion locus is usually performed using PCR and Sanger sequencing the flanks and preinsertion locus and as with the structure of the insert, NGS can help when defining the insertion site. With regards to point iii), data on the presence of the insert(s) over several generations is usually produced with Southern analysis. Provided that there is sufficient coverage at the site of insertion(s), the use of NGS methods to identify all the junctions within the genomic DNA could substitute the need for Southern analysis also for this data requirement. With regards to point iv), data on the expression of newly expressed proteins is usually obtained by measuring the protein levels by ELISA/ Western blot. However in a number of cases, Northern analyses or RT-PCR may also be needed and NGS-based methods (e.g RNAseq) may be a good alternative. At present, the main uses are in the characterisation of the insert(s) and insertion site(s) but there is potential for further applications such as in characterising the expression of the inserted sequences. In order to evaluate the quality of the analyses, information on the sample preparation and methods used as well as on the sensitivity reached, are key and should be provided.

Katia Pauwels, delegate from Belgium to the GMO Network (MC/FF), gave a talk on the high throughput DNA sequencing and its possible added value in the risk/safety assessment of GM plants. The talk was focused on summarising the conclusions from the 'Next-generation sequencing as a tool for the molecular characterisation of GMOs' workshop held in Brussels on 25 November 2013 and from the OECD workshop on 'High-throughput DNA sequencing in the Safety Assessment of Genetically Engineered Plants' held in Paris on April 18 2016. Examples of GM applications submitted in the EU were discussed where NGS methods were used to molecularly characterise GM crops. The potential of NGS to detect small insertions as well as its increased cost-effectiveness especially for stacks with high number of inserts were considered as aspects offering added value to the current risk assessment process. Its potential to provide information on the expression of both intended and unintended inserted /modified sequences by e.g. RNA sequencing and transcriptome profiling was also pointed out. The

possible use of NGS in new plant breeding techniques such as cisgenesis/intragenesis and targeted mutagenesis was also discussed. A number of technical bottlenecks of the NGS technology were also discussed such as the difficulties in accurately assembling the entire T-DNA insertion. From a risk evaluator's perspective, assessing the various bioinformatics tools and different parameters that may lead to different results as well as the increased need for high performance computing and storing capacity will be challenging. These elements, together with the amount of available information on reference genomes and raw data, as well as the choice of analysis and visualisation software, are important aspects for the evaluation of NGS-derived data in a risk assessment frame.

The presentations were followed by a general discussion.

A delegate from Hungary asked whether NGS could help to distinguish between natural and targeted mutations. Mr Casacuberta replied that although this would be a very relevant question it may not be easy to answer considering that (i) reported reference genomes are not complete (ii) the genome sequence for the variety used will probably not be available and, as genetic variation among varieties is high (e.g. for maize varieties could be as high as 50%), the use of the species reference genome sequence is limited. In addition, irrespective of the power of NGS technologies, it may not be possible to distinguish between mutations resulting from off-target effects as a result of genetic modification and spontaneous mutations. The delegate from Hungary also asked if the NGS technology could be used to identify unintended effects. Mr Casacuberta replied that at this point the limitations of the data and the methods available make NGS of limited value for this purpose.

A delegate from Austria commented that guidelines on the use of NGS methods are welcomed and they would be considered helpful for risk assessors. In addition, considering that there are many available bioinformatics tools for the analysis of NGS data that may give different results, there is a need for standardisation and therefore providing guidelines would help in the assessment of such data. He also mentioned that based on the most recent literature, NGS methods may produce data with an error rate between 0.1 – 15 %; this is considered high and could have profound relevance in the risk assessment process. Mr Moxon replied that the tools used by applicants in submitted applications are considered standard but the actual parameters used can vary considerably. The greatest need therefore for a standardised workflow could be the harmonisation of these parameters. Concerning the reported error rate of up to 15%, Mr Moxon clarified that such high error rates would probably be reported for methods using very long reads (a few kb) and there are currently substantial efforts in the scientific community to reduce this error rate. The Illumina technology for example is not expected to produce such error rates.

A delegate from Denmark asked if using different programs results in a different outcome or in a difference of the covered sequence. Ms Pauwels replied that having the right bioinformatics tools is important for both, particularly when large inserts are analysed, although there has not been such a case in the applications submitted so far. Mr Moxon replied that the analysis pipeline is generally similar but one could occasionally get different answers, especially in

approaches where a reference genome is used to map reads. Therefore using the right parameters could be very important and having guidelines would be useful both for applicants and risk assessors.

A delegate from Poland commented that the presented information was considered very useful and that a thorough evaluation on the possible NGS applications should be conducted. For instance, ”target capture” sequencing is a different approach to whole genome sequencing and could be used for junction detection. Mr Casacuberta replied that the potential for NGS-based methods is evident but it is important to define the questions that need to be answered before looking into the available NGS tools that can be used to answer them.

A delegate from Austria asked if the use of NGS could be applied in the frame of NBTs where a GMO may be defined by the 30-nucleotide threshold. Mr Casacuberta replied that the level of genome variability between individuals and varieties is large and to define a threshold of change between two varieties may be very difficult, but checking for e.g. this 30 bp change using NGS may be useful.

A delegate from Denmark commented that a technical threshold of 20-25 nucleotide change derived from statistical analysis on the likelihood of spontaneous DNA changes is currently being discussed in relation to new breeding techniques such as cisgenesis and subcloning.

EFSA thanked the Member State delegates for the positive feedback on its proposal to produce guidelines on the use of NGS technology and also mentioned the technical difficulties in receiving and storing raw data produced by NGS methods. Currently, this is an unresolved issue and EFSA is working together with EC’s Joint Research Centre (JRC) to determine the best solution.

4.9 Gene drive and potential implications for environmental safety; gene drive in malaria mosquitoes

Boet Glandorf, delegate from the Netherlands to the GMO Network (ERA), gave a talk on the policy report of the Dutch National Institute of Public Health and the Environment (RIVM) on the ‘gene drive’ technology and its implications for the environmental risk assessment. Ms Glandorf also presented the main conclusions from the Workshop on gene drive in malaria mosquitos financed by the FNIH and Bill & Melinda Gates foundations held in May 2016 in Washington. The first part of the presentation focused on the scientific principles behind this approach and was followed by the potential applications for humans, agriculture and the environment. The third part focused on the potential risks of this technology.

Gene drive is an approach used to ‘drive’ genes into a population. Using CRISPR technology, gene systems can alter or supress any sexually reproducing populations. Gene drive can be very effective with fast reproducing organisms such as insects and can therefore help fight tropical diseases such as malaria. Besides insects, gene drive technology can also be applied to plants and animals e.g. immunising disease-prone animals or making plants tolerant to pesticides. Given the great potential of this technology, discussions on evaluating the associated potential risks have already started and the RIVM published a policy report on this topic focusing on risks associated with this method for the environment. Gene drives are fast, consequences can potentially be irreversible and effects can be population-wide. As of now, current knowledge and GMO assessment methods are thought to be sufficient in properly evaluating the

potential environmental risks although it is acknowledged that different expertise may be necessary for adequate assessment. Several national and international activities are currently ongoing to collect relevant information and identify important data gaps and need for expertise.

The presentation was followed by a general discussion.

EFSA asked if the existing EFSA guidance document related to GM insects was already considered in the workshop in Washington. Ms Glandorf replied that it was not considered because the presented work was an independent exercise but it will probably be taken into account in the next stages of the activity together with other existing relevant information.

A delegate from Belgium noted that gene drive efficiency varies a lot from one organism to another, depending on the speed of reproduction and the efficiency of the homology-directed repair. He also mentioned that sometimes working with CRISPR systems generates unintended gene drive. Ms Glandorf replied that because of the low efficiency of homology-directed repair in plants, there are low chances of creating efficient gene drive in plants.

A delegate from Slovenia commented on the importance of post-release monitoring of insects obtained by gene drive, in order to observe any unintended effects. Ms Glandorf agreed that monitoring is important, and noted that the risk assessment of these insects could be similar to that of GM mosquitoes, for which there is already some experience.

A delegate from Poland commented on the potential of this technology in improving human health by e.g. tackling the problem of malaria. He also mentioned that possible side-effects of such genetic modifications should be taken into account and expressed concerns on the lack of expertise for post-release monitoring.

A delegate from Finland commented that there is a need for proper tools to support the risk assessment of gene drive and CRISPR/Cas in general, to evaluate if these genetic changes create unintended effects and whether they affect fitness. She also expressed her opinion on the apparent lack of expertise in assessing how such genetic modifications might affect the fitness of the organism.

A delegate from France informed that there is on-going work at the national level to provide information on the use of GM mosquitoes and Wolbachia-infected mosquitoes as vector control, to collect information on sterile insect techniques, and to elaborate criteria for risk assessment. She also expressed her interest to collaborate with RIVM. Ms Glandorf replied that the Dutch institute is open to all collaborations and would happily share all the information gathered so far.

A delegate from Ireland asked if there were discussions concerning the public awareness on the gene drive technology during the workshop on gene drive in malaria mosquitoes. Ms Glandorf replied that the workshop was more focussed on scientific aspects. However in the workshop it became clear that researchers are aware of the importance of involving the public before using this kind of applications.

A delegate from Germany asked whether there was a possibility for the parasite to find alternative vectors. Ms Glandorf mentioned that this issue was identified during the discussions in the workshop. A member of the GMO Panel's ERA WG pointed to the EFSA Guidance for environmental risk assessment of GM animals, where the issue brought up by the German delegate is extensively analysed.

5. Any Other Business

5.1 Upcoming events

Elisabeth Waigmann reminded the GMO Network experts of the upcoming events such as the public consultation periods for the allergenicity and the LLP GDs (expected to start in late summer and autumn of 2016 respectively), as well as the Info session on the allergenicity GD meeting at the end of 2016 in Parma.

6. Date for next meeting

EFSA proposed to have the next GMO Network meeting in May 2017.

7. Closure of the meeting

The Chair thanked the speakers and the participants for the active and fruitful discussions.

References

Codex Alimentarius, 2003. Codex principles and guidelines on foods derived from biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome.

Codex Alimentarius, 2009. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Annex III: Food safety assessment in situations of low-level presence of recombinant-DNA plant material in food.

Devos Y, Romeis J, Luttik R, Maggiore A, Perry JN, Schoonjans R, Streissl F, Tarazona JV and Brock TC, 2015. Optimising environmental risk assessments: Accounting for ecosystem services helps to translate broad policy protection goals into specific operational ones for environmental risk assessments. EMBO Reports, 2015, 16, 1060-1063, doi: 10.15252/embr.201540874

Devos Y, Gaugitsch H, Gray AJ, Maltby L, Martin J, Pettis JS, Romeis J, Rortais A, Schoonjans R, Smith J, Streissl F, Suter GW II (2016). Special issue: Advancing environmental risk assessment of regulated stressors under EFSA's remit. EFSA Journal, 14(S1):s0508, doi:10.2903/j.efsa.2016.s0508

EFSA (European Food Safety Authority), 2015. Clarifications on EFSA GMO Panel recommendations on the Insect Resistance Management plan for genetically modified maize MON 810. EFSA Supporting Publication 2015; 12(7):EN-842, 14 pp. doi:10.2903/sp.efsa.2015.EN-842

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012. Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810. EFSA Journal 2012;10(12):3016, 32 pp. doi:10.2903/j.efsa.2012.3016

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Scientific Opinion updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to Bt-maize pollen. EFSA Journal 2015;13(7):4127, 31 pp. doi:10.2903/j.efsa.2015.4127

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016. Scientific opinion on the annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2014 from Monsanto Europe S.A. EFSA Journal 2016;14(4):4446, 26 pp. doi:10.2903/j.efsa.2016.4446

EFSA Scientific Committee, 2016a. Scientific opinion on coverage of endangered species in environmental risk assessments at EFSA. EFSA Journal 2016;14(2):4312, 124 pp. doi: 10.2903/j.efsa.2016.4312

EFSA Scientific Committee, 2016b. Scientific opinion on recovery in environmental risk assessments at EFSA. EFSA Journal 2016;14(2):4313. 85 pp. doi: 10.2903/j.efsa.2016.4313

EFSA Scientific Committee, 2016c. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. doi:10.2903/j.efsa.2016.4499

Hofmann F, Otto M and Wosniok W, 2014. Maize pollen deposition in relation to the distance from the nearest pollen source under common cultivation – Results of 10 years of monitoring (2001–2010). Environmental Sciences Europe, 26, 24, doi:10.1186/s12302-014-0024-3

Hofmann F, Kruse-Plass M, Kuhn U, Otto M, Schlechtriemen U, Schröder B, Vögel R and Wosniok W, 2016. Accumulation and variability of maize pollen deposition on leaves of European Lepidoptera host plants and relation to release rates and deposition determined by standardised technical sampling. Environmental Sciences Europe, 28, 14, doi:10.1186/s12302-016-0082-9

Lang A, Oehen B, Ross J-H, Bieri K and Steinbrich A, 2015. Potential exposure of butterflies in protected habitats by Bt maize cultivation: A case study in Switzerland. Biological Conservation, 192, 369–377

Rabinowitz D, 1981. Seven forms of rarity, In Biological aspects of rare plant conservation (ed. H. 4611 Synge), pp 205-217. John Wiley & Sons, Chichester, UK.