GMO UNIT

Minutes of the 2nd Meeting of the EFSA Scientific Network for Risk Assessment of GMOs[1]

held in Parma on 9-10 June 2011

European Food Safety Authority[2] (EFSA), Parma, Italy

This report reflects the discussion and comments made at this meeting. This report has been subjected to verification by the intervening participants. This report is not and cannot be regarded as representing the position, the views or the policy of the European Food Safety Authority or of any national or EU Institution, agency or body.

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[1] Question No EFSA-Q-2011-00297
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PARTICIPANTS

GMO Network: The GMO Network Member Organisations from 24 EU Member States and Norway (see http://www.efsa.europa.eu/en/gmo/gmonetworks.htm) appointed in 2010 through the EFSA Advisory Forum 44 experts as delegates to attend the GMO Network meetings. 36 Experts attended the 2nd meeting. As observers, 4 experts from Candidate Countries attended the meeting.

GMO Unit: Per Bergman (HoU, Chair), Yann Devos, Zoltan Diveki, Karine Lheureux, Yi Liu, Sylvie Mestdagh, Claudia Paoletti, Nancy Podevin, Stefano Rodighiero, Reinhilde Schoonjans and Elisabeth Waigmann (DHoU, co-Chair).

European Commission: Kaja Kantorska (DG SANCO).

Invited experts: Christer Andersson, Evert Jacobsen, Gijs Kleter, Harry Kuiper, Howard Davies, Jeremy Sweet, Jörg Romeis.

See the participants list in Appendix 1 and apologies.

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1. WELCOME BY CHAIR

Per Bergman, Head of the EFSA GMO Unit, opened the meeting by welcoming the participants to this second meeting of the EFSA Scientific Network for risk assessment of GMOs (hereafter referred to as "GMO Network" or "Network"). Networks are established for all sectors of EFSA to provide a forum for national experts to meet once a year to discuss harmonisation of risk assessment practices and to exchange information. He thanked the Member State experts for their support in building a good communication between risk assessors in Europe.

2. TOUR DE TABLE AND APOLOGIES FOR ABSENCE

All participants presented themselves, their background and affiliation during a tour de table. Apologies for absence were received from 2 experts.

3. ADOPTION OF THE AGENDA

The draft agenda was prepared by EFSA following recommendations of the GMO Network after the first meeting. The aim is to focus discussions on fewer topics, to deepen the discussions on previous topics and to receive feedback from MS on ongoing EFSA mandates. One half day is focussed on comments on applications, recurrently submitted to EFSA by MS during risk assessment, and that would merit a discussion to achieve better common understanding. The draft agenda as published at the EFSA website http://www.efsa.europa.eu/en/events/event/110609.htm was adopted.

4. DECLARATIONS OF INTEREST

Representatives of the organisational members of the GMO Network and their alternates were asked to fill in an Annual Declaration of Interest (ADoI) to declare any interest that might be considered prejudicial to their independence. In accordance with EFSA's Policy on Declarations of Interests, EFSA screened the received ADoIs. Since representatives of the GMO Network are nominated by Member States, no conflicts of interests are expected for the nature of the activities of the GMO Network.

In accordance with the EFSA management board decision on the rules and procedures of EFSA Networks, the representatives of the organisational members of the GMO Network, their alternates, observers and staff of the European Commission were requested orally at the beginning of the meeting to declare any interests that might be prejudicial to their independence in relation to the items on the

agenda. With regard to this meeting no other interests than those already declared in the ADoIs and screened by EFSA in accordance with its Policy on Declarations of Interests and implementing documents thereof, were declared by the experts.

5. MINUTES OF THE PREVIOUS NETWORK MEETING

The draft minutes of the 1st meeting of the GMO Network (held on 22-23 November 2010) were presented to the Network shortly after the meeting, and participants were given the possibility to verify that their views expressed were correctly reflected. After amendments, the minutes were adopted and published on the EFSA website: http://www.efsa.europa.eu/en/events/event/gmo101122.htm.

6. PRESENTATIONS BY EFSA TO UPDATE THE NETWORK ON

6.1. EFSA work and active mandates of the EFSA GMO Panel

Mandates of the GMO Panel and GMO Unit include GMO applications, risk assessment guideline development, requests for scientific advice, contracts etc. The GMO Network was provided with an overview table of the active mandates, highlighting the ones for discussion during this meeting.

6.2. Follow-up of the 1st meeting

Regarding 90-day feeding studies with whole food and feed, the French delegate Chantal Arar and the Chair of the GMO Panel Harry Kuiper provided feedback and gave short presentations on the status of their respective Working Groups. The work of ANSES has been published and the outcome of the report was being duly considered during the work of EFSA that was still ongoing. An expert from ANSES was taking part of the EFSA Working Group.

6.3. Sciencenet (trouble shooting and short training)

Participants have successfully used the secured online tools for document sharing: the Information Exchange Platform (IEP) for all EFSA national contacts and the more specific Sciencenet for EFSA GMO Network members. The GMO Unit offered further support to access documents online and demonstrated the electronic discussion forum for GMO Network experts.

7. BREAKOUT SESSIONS: DISCUSSIONS WITH THE NETWORK ON MS ACTIVITIES RELATED TO ACTIVE MANDATES OF THE EFSA GMO PANEL

The EFSA GMO Unit scientists had prepared background information on the various topics and relevant documents. The GMO Network delegates received in advance of the meeting this background information as well as questions for guiding the scientific discussion on risk assessment and for supporting all participants to express their view on the topics.

7.1. 7a. MC Breakout session on Cisgenesis

The Chair and an EFSA GMO Unit scientist introduced the break-out session on cisgenesis with the Molecular Characterisation (MC) and Food/Feed (FF) safety experts.

Introduction

Different activities on new techniques for introducing new traits in plants (including cisgenesis) are ongoing in the European Union. Firstly, a Working Group of the European Commission and the Member States (EC-MS WG) investigates if new techniques fall under the GMO legislation. Secondly, the Institute for Prospective Technological Studies (IPTS) and the Institute for Health and Consumer Protection (IHCP) of the Joint Research Centre (JRC) reviewed for the EC the state-of-the-art of these new technologies, their level of development, their current adoption by breeders and

prospects for a future commercialisation of crops based on them[3]. Thirdly, EC asked EFSA whether the existing guidance on risk assessment should be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques (incl. cisgenesis), and what the risks in terms of impact on humans, animals and the environment that the eight techniques listed could pose, are, irrespective of whether or not they fall under the GMO legislation.

Presentation by Professor Evert Jacobsen

The present EU GMO legislation was developed at the time when the inserted genes primarily originated from a species other than the host species (e.g. transgenes from bacteria in GM host plants). Cisgenesis differs from transgenesis since the inserted gene originates from the same species as the host species. Moreover, it was clarified that selection of cisgenic plants can be done without using a marker gene. T-DNA/P-DNA borders were described as unlikely to cause unintended changes in the GM plants as indicated in reports by COGEM and others; and different plant species have been shown to contain T-DNA border like sequences. Regarding the stability of the inserted sequences, it was mentioned that the Distinctness Uniformity Stability (DUS) criteria for variety right approval include stability.

Plant breeding techniques evolved from crop domestication, over classical breeding to mutation breeding. Comparisons were made between cisgenesis and traditional breeding, focussing on possible unintended effects and describing genome changes that take place in traditional breeding (e.g. translocations, transposons, somaclonal variation), as well as in cisgenesis (e.g. random insertions). Until now, plant breeding did not lead to major safety problems for humans or animals and is therefore considered to have a history of safe use. A key message was that plant genomes are dynamic and have a large buffering capacity to accommodate changes. Small changes therefore do not necessarily result in undesired effects. Regarding position effect on gene expression, it was mentioned that breeders address this issue during the normal selection procedures in the field. It was noted that allelic variation of expression is more important than allelic variation of coding sequence.

Allergens and toxins are known to be present in many domesticated crops and are checked at variety registration. Self-monitoring of toxic compounds is applied by breeders if needed (e.g. glycoalkaloids in potato). Variation in the levels of metabolites between plants of the same species can be substantial.

The finding that the level of unintended effects due to cisgenesis is the same as for classical breeding by crossing plants and less likely than for newer breeding techniques such as mutation breeding[4], underlies the proposal of the US Environmental Protection Agency (EPA) to exempt cisgenic plants from registration[5]. Prof. Jacobsen concluded that strategies for safety assessment of traditionally bred plants can be used for cisgenic plants as the same gene source is used.

Discussion on the technology

Prof. Jacobsen clarified to the Finnish delegate that functional T-DNA borders do not need to be in original form from *Agrobacterium* and that different *Agrobacterium* strains use different T-DNA sequences. The Austrian delegate agreed that *Agrobacterium* mediated transformation is relying on a natural process, but pointed to the fact that biolistic transformation is completely artificial with unpredictable side effects and asked for a comment on the latter aspect. Prof. Jacobsen acknowledged that unintended effects are more likely when biolistics are used.

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[3] Report available at http://ipts.jrc.ec.europa.eu/publications/pub.cfm?id=4100

[4] National Academy of Sciences, USA, 2004 "*Safety of Genetically Engineered foods: Approaches to assessing unintended health effects*" Figure 3-1 at page 64
http://www.nap.edu/openbook.php?record_id=10977&page=64#p2000a7b39960064001

[5] Federal Register / Vol. 76, No. 51 / Wednesday, March 16, 2011 / Proposed Rules page 14358
http://edocket.access.gpo.gov/2011/pdf/2011-5997.pdf

The Danish delegate agreed that cisgenic plants do not pose additional hazards compared to traditional plants. Intragenic plants or cases where alternative promoters would be used are not addressed here and the importance to take a clear step-by-step approach was underlined.

The Polish delegate pointed to the ever increasing gene variation in nature and that, in breeding through crossing, many genes are transferred in an unknown manner. Prof. Jacobsen acknowledged this and added that all genes available to the traditional breeder should be allowed for cisgenesis.

The Hungarian delegate recalled that the functionality of all parts of the genome has not been identified and therefore the stability of the trait needs to be checked. Prof. Jacobsen assured this is part of variety right registration.

In addressing the safety question of the Czech delegate, it was mentioned that potential novel fusion proteins at the insertion sites would be linked to the same safety considerations as for those created by traditional breeding e.g. translocation breeding.

The Croatian, Irish, Maltese and Polish delegates made statements on the legislative framework, respectively as follows: the EU legislation addresses many issues that are linked to the origin of the transgene in transgenesis rather then to the genetic modification technique as such; cisgenic plants might not fall under the Biosafety Cartagena protocol; cisgenesis might fall under Novel food regulation; and in other parts of the world (e.g. Canada) regulation of novel plants depends on the trait. These statements are informative for EU risk managers.

Work on a cisgenic potato case study for risk assessment

To focus the scientific discussion on relevant questions for risk assessment, a prepared case study was sent to the GMO Network prior to the meeting. In short, it consisted of a variety potato in which an resistance (R) -gene from another commercial potato was introduced. The provided information in the application was based on Directive 2001/18/EC, on DUS requirements and complemented with compositional analyses.

In response to the Hungarian delegate, questioning why conventional breeding was not used, it was clarified that the time consuming introduction of 5 different alleles (to obtain durable resistance) by crossing would produce a potato that is no longer edible because of the linkage drag. The Czech delegate confirmed that breeding, certainly when using wild relatives, often results in negative phenotypes. It was recalled that potato infection with late blight (the oomycete *Phytophthora infestans*) created problems already 150 years ago in Ireland and since then there has been no solution found by breeding. Current solutions involve spraying chemicals that may turn out not to be safe.

Flanking sequences

Given natural rearrangements in the genome and absence of health effects thereof, the Danish delegate stated that using all transgene risk assessment criteria for this cisgenesis case, would result in overregulation. It should be discussed whether certain data can be omitted, for instance the information on the flanking sequences. Instead Hungarian and Austrian delegates indicated that flanking sequences are essential since informing on the insertion place and the possible interruption of endogenous genes. Others delegates propose to relate the need for this data to health concerns that could be caused by cisgenesis whilst being compared to traditional breeding.

Protein expression data

Given the natural variation in gene expression amongst different varieties, the Czech delegate found insert expression data gathered from two seasons excessive. Assessment of trait stability however is more important, for instance in case of stress tolerance involving expression of up to 1500 genes. The Hungarian delegate agrees that gene expression may not be relevant but protein expression levels must be asked. The Dutch delegate indicated that the presence of the gene product in our current food/feed (i.e. current exposure) would be a key element to be considered in the risk assessment.

Allergenicity & Toxicity

The Finnish delegate pointed out that for food crops, that are rarely, and if, mostly weakly allergenic, e.g. the current potato case, bioinformatics analyses should be sufficient. In reference to unintended effects from an example outside this discussion[6], the Hungarian delegate advocated toxicological studies with whole food. In the view of the Dutch delegate, sacrificing animals for testing food should not be allowed unless potential unintended adverse effects have been identified. The Irish delegate added that absence of unintended effects cannot be proven for any of our food. The Italian delegate agreed that when a protein from food is already eaten and is transferred into the same species, there is no need for toxicity studies. The French delegate was interested in the topic of possible toxic effects due to the natural changes in the genome as dealt with by traditional breeders.

The MS experts reflected if the above requirements would be altered in the case where the cisgene would originate from a wild relative not normally consumed, but used by plant breeders.

The Spanish delegate indicated that the bioinformatics searches with the allergen database should be sufficient in case experts deem that these databases are well developed. Multiple delegates indicated that, concerning the possible toxic effects of the cisgenic protein, it is important to assess the knowledge on this protein.

Field trials and compositional analysis

The German delegate expressed the view that the number of field trials should be in line with current EFSA guidance (i.e. 8 replicated sites) while others deemed less (e.g. 4 replicated sites) sufficient. The Czech delegate proposed that the sites of field trials should be in countries growing the plants commercially. Concerning the analysed compounds, the Danish delegate noted that the OECD list is not only for GM plants, but also for new varieties.

Closing remarks

Different delegates confirmed that it is important to distinguish between “need to know” and “nice to know” information. The breeders do take the responsibility to make sure that no harmful food is produced. Whether or not cisgenesis falls within the same risk area as transgenesis was discussed in detail. Similarities with non-regulated bred plants were found as well as similarities with transgenic plants. For the latter similarities, areas have been identified where many judge that risk assessment data requirement could be relaxed.

7.2. 7b. ENV Breakout session on PMEM of GM plants

The co-Chair and an EFSA GMO Unit scientist introduced the break-out session on Post-Market Environmental Monitoring (PMEM) to the Environmental (ENV) risk assessment experts.

Introduction

Active mandates received from the EC for the EFSA GMO Panel on PMEM included the update of the 2006 PMEM scientific opinion and the assessment of annual reports on PMEM of GM crops currently grown in the EU. A public consultation had been held on the draft updated scientific opinion on PMEM, and the summaries of key comments were shared with the GMO Network. The following discussion had the objectives to (1) to encourage the Member States not having responded to the public consultation to raise outstanding comments, if any; and (2) to discuss the possibility of using existing biodiversity networks or programmes to monitor GM plants under general surveillance (GS).

The Belgian delegate questioned if a discussion on general surveillance networks, being seen as a management issue in Belgium, falls within the remit of the GMO Network. The Chair clarified that

[6] GM Pea (with a bean transgene) that failed safety checks during its development

EFSA has been mandated by the EC to work on PMEM. The discussion with the GMO Network aims to complement information on how to obtain annual PMEM reports that could further inform the Environmental Risk Assessment (ERA), e.g. regarding long-term effects. Hence the direct interest of EFSA and its GMO Panel responsible for ERA. Recognizing this feed back loop, the EC has mandated the EFSA GMO Panel to assess the annual reports on PMEM of GM crops grown in the EU.

Member States' views on the draft scientific opinion on PMEM

The Danish delegate expressed concerns about the feasibility of GS of GM plants and highlighted the limitations of the farmer questionnaires to collect biodiversity-related data. The Slovenian delegate questioned the relevance of a proper PMEM plan in absence of a suitable non-GM comparator. The Irish delegate explained that, in absence of commercial cultivation of GM crops in Ireland, they did not comment the draft scientific opinion on PMEM due to lack of experience. The French delegate welcomed the draft scientific opinion and the good framework for PMEM in the EU outlined therein, in particular specific recommendations made by the EFSA GMO Panel such as the suggested set-up of a cooperative EU monitoring of agro-ecosystems to survey various stressors (e.g. GM plants, pesticides). The French delegate welcomed the proposal by the EFSA GMO Panel to build an active cooperation between applicants and Member States. The Austrian delegate explained that, in some cases, relevant data are not published in peer-reviewed literature and therefore all sources of information should be considered. He thereby supported the draft guidance of EFSA referring to conference reports, research studies reports, etc. as sources of information. Some delegates questioned the proposal by the EFSA GMO Panel to Member States for setting-up centralised reporting centres.

Use of Member States' existing environmental monitoring networks for mandatory PMEM

The EFSA GMO Panel expert reminded that, according to the EU regulatory framework, applicants have the legal obligation to submit a PMEM plan as part of their applications for GMO commercialisation. A plan for GS is mandatory, aiming at detecting any adverse effect on human health and the environment that has not been anticipated by the ERA. The first objective of GS is to detect a change in the agro-ecosystems and this objective is independent from GMO cultivation. Against this background and according to the EU regulatory framework[7], existing networks for biodiversity surveillance (e.g. surveillance of fauna: butterflies, birds), and surveys of inputs in cropping systems (e.g. pesticides, seeds) at local/regional/national level are seen as helpful tools to detect a change in trend in our agro-ecosystems. These networks, where available, monitor protection goals. They are likely to collect data of interest to establish relevant temporal and spatial baselines and are therefore likely able to detect a change, if occurring.

When drafting the EFSA scientific opinion on PMEM, little information was available on the aforementioned networks. Issues in need of clarification were: what type of data do they collect; are these data available; what is the format of these data; are these networks willing to be associated to GS of GM plants? These questions were shared with the GMO Network prior to the meeting and the Chair invited the delegates of the Member States to address them in a tour de table.

Networks monitoring relevant protection goals (e.g. flora & fauna conservation, water quality, soil functionality) and indicators, were confirmed to be active in various Member States like in Austria, Bulgaria (e.g. plants, birds and bugs), Denmark, Estonia (e.g. birds), Finland and Sweden (e.g. birds and flora), France (e.g. insect pests, diseases, flora), Ireland (e.g. arthropods), Poland (e.g. butterflies & birds, pests & diseases in arable land, changes in agricultural landscape, protected mammals & plant species, water & soil quality), Slovakia (e.g. agricultural & environmental parameters), Slovenia (e.g. Natura 2000 programs), The Netherlands (e.g. butterflies, plants, protected species) and UK (e.g. countryside survey, birds, butterflies, water quality etc.). Some delegates (e.g. from Ireland, France)

[7] Commission decision supplementing Annex VII of Directive 2001/18/EC

highlighted the need for a proportionate monitoring of agriculture as a whole, not only focusing on GMOs. In this respect, existing surveillance networks can be looked at in terms of protection goals, assessment and measurement endpoints, indicators, temporal and spatial scale.

Various delegates were sceptical about the usefulness of these networks for general surveillance of GM plants. Most of the delegates pointed to the numerous limitations of the existing monitoring networks, for example the limited funding (e.g. Estonia, Slovenia), the networks' dependence on volunteers (e.g. The Netherlands), the limited access to raw data, the type of data collected, the different spatial and temporal scales of data collection (e.g. Austria), the difficulty to adapt these networks for general surveillance of GM plants and the challenge to coordinate different existing networks (e.g. Finland). The UK noted that existing networks represent a MS's policy on agri-environmental monitoring and GMO monitoring should be considered in this context. With this in mind the UK's advisory committee has a working group that was established to consider the adequacy of Existing Surveillance Networks (ESNs) to detect levels of change associated with GM crop production taking into account the spatial distribution and uptake of these crops. Preliminary results demonstrate that the use of ESNs for GM crop surveillance in the UK is plausible. However, there are challenges linking a change to a particular GM crop. The working group is likely to publish its report early next year.
In order to overcome the high variety of receiving environments and associated production systems, it was proposed to set minimal data requirements related to general indicators at the EU level, whereas data requirements for specific indicators could be set at national level. In addition, bearing in mind that such networks might not always be available, alternative tools need to be explored. As an illustration, the French delegate briefly presented the national project to survey and record a range of environmental parameters under different cropping conditions (e.g. flora surveillance ongoing from 2002). The Austrian delegate recalled that PMEM comes after the ERA and after risk management measures, if any. Well designed studies (likely under case-specific monitoring) should provide robust and statistically-significant data that may feed into the ERA. The Austrian delegate continued by stating that long-term effects could hardly be anticipated and properly assessed during the ERA. Therefore, general surveillance is needed to detect possible adverse effects that were not anticipated during the ERA and thus to cover this lack of knowledge or data. He recognised the limitations of the networks and invited the national competent authorities to consider alternatives to get the data needed. A solution would be to monitor specific parameters, but general surveillance is not a targeted monitoring as unanticipated unintended effects are to be considered.
The Belgian delegate invited EFSA to consider the outcome of the EC Working Group on PMEM, which already assessed the suitability of existing EU networks.

Closing remarks

A majority of the delegates was of the opinion that the use, selection and adaptation of networks should be left to the discretion of each Member State in the light of the relevant protection goals. However, further guidance on assessment criteria for the suitability and adaptability of existing networks from EFSA would be helpful.

8. PLENARY SESSION: DISCUSSION WITH THE NETWORK ON MS COMMENTS SUBMITTED ON APPLICATIONS, RELATED TO RISK ASSESSMENT OF POTENTIAL INTERACTIONS IN STACKED EVENTS

The co-Chair and EFSA staff scientists opened the session by summarising the case study that had been shared with the GMO Network in preparation of this discussion. While comments received from Member States on applications are mainly critical and call for more data to be added to the submitted dossier, the majority of MS do not submit comments whenever they are satisfied with the dataset or when holding the view that less data would be sufficient. One aim of the discussion was to offer an opportunity for MS to share with each other and with EFSA all views on this case study dataset.

The case was a 4-stacked maize for which the 4 single events as well as both parental 2-stacks were previously assessed and concluded as safe. The scope of the application was limited to import and processing excluding cultivation. The topic of discussion was the risk assessment of potential interactions in this 4-stacked maize and the recurrent MS comments received on the dataset submitted by the applicant. The received MS comments had been grouped as regarding the data for 1) Molecular characterisation, 2) Field trials, 3) Compositional analysis, 4) Assessment of interaction and 5) Allergenicity. The GMO Network was asked to inform if the dataset provided was considered sufficient to conclude the risk assessment. In case the MS would like to see more, less or different data, they were asked to substantiate their proposal with scientific arguments. In such cases the EFSA GMO Unit scientists asked about the underlying problem formulation and why that problem could not be assessed with the data available.

The Danish, Irish and Dutch delegates shared that when no comments are received from them on a dataset, it should be interpreted that the dataset was satisfactory for concluding the risk assessment.

General remarks on assessment of stacks and potential interactions

The Danish delegate is of the opinion that stacked events should not be covered by the GM regulation, since they are the product of natural breeding of already assessed single events and no problematic interactions are expected. Moreover, interactions between gene products are happening all the time when different foods are mixed (DNA, proteins and other components) during cooking. The Dutch delegate supported this view as in traditional breeding different genetic backgrounds can be combined, also resulting in potential interactions. Confident assessment of the single events is enough, together with the demonstration of the safety of the inserted proteins. The Irish delegate added that the natural variations in a dynamic plant genome trigger also potential interactions and natural changes.

Molecular Characterisation

Potential interactions at the DNA level were assessed by analysing the integrity of the inserts in the stack through Southern analysis. The Austrian delegate was of the opinion that the evaluation was complicated by poor visual quality of the blot, by inappropriate choice of molecular weight markers and inappropriate choice of restriction enzymes generating too large fragments. Finland responded that these Southern blots were deemed satisfactory and are usually noisy.

Stability of the trait should, according to the Hungarian delegate, not only be tested on F1 generation plants but also on further generations. It was clarified that generally GM maize is marketed as F1 hybrids produced each season newly from their parental lines. Thus, there is no need to demonstrate stability over several subsequent generations. In this case the Hungarian delegate agreed that testing on F1 maize plants is sufficient.

Potential interactions at RNA level (e.g. RNA silencing) were assessed through analysis of the levels of newly expressed proteins in the stack and the respective single events. Since these levels were comparable, no indication for interactions was found. This triggered no further discussion with the GMO Network.

Potential interactions at protein level were assessed through analysis of synergistic/antagonistic effects in insect bioassays on target insects. The Belgian delegate asked why such studies were provided in an import and processing application; the Spanish delegate asked about the connection of the insect bioassays and food/feed safety. The GMO Panel Member clarified that these studies have no connection to human toxicity, but are a useful readout to detect interactions between the newly expressed proteins that would alter their functionality. It was added by an EFSA GMO Unit scientist that therefore the study provides additional information on potential interactions, in addition to the protein expression levels. The Hungarian delegate added, and the GMO Panel expert agreed, that the bioassay is useful to prove that the original function of the protein is maintained.

Field trials

The field trials for this case were conducted outside of the EU and were accepted because the scope is for import & processing and their design fulfilled the requirements specified in the EFSA guidance. While the Danish delegate was satisfied that trials are carried out in countries where the plant is cultivated, he remarked that in cases of awareness of different environmental condition (climates and soil) it would perhaps be better to test in 2 sites and 2 seasons. He continued that, since the climate cannot be controlled, providing more guidance in this area is difficult. The Austrian delegate found that the trial should cover also diseases, pest and herbicide practice (spraying) of the stack, as it is done nationally when new varieties are tested in Austria over 2 years, 2 seasons and 8 different sites. The Hungarian delegate asked for clarification on which herbicides were sprayed in the trials and the Belgian delegate advocated that when the single events have been well assessed, sprayed and unsprayed, then the 4-stack not necessarily needs to be sprayed.

Compositional analysis

Compositional analysis was used to identify unintended effects, e.g. such that are due to interactions in the stacked event, and was performed according to OECD standards although the EFSA GMO Panel is aware of suggestions to test more compounds. The Hungarian delegate advocated that proteomics should be applied to assess unintended effects (including interactions) and to show that the proteins expressed are identical to the original ones and that no new proteins are expressed. This was contested by the Czech delegate since proteomics is still too complex to allow good data interpretation for risk assessment (generation of data on thousands of interactions). The Danish delegate was satisfied that the presence of the newly expressed proteins had been assessed for the single events.

While the Austrian delegate was satisfied that currently the composition is analyzed according to OECD, the Belgian delegate informed that some compounds such as dietary fiber and carbohydrate fraction were suggested to be added to the OECD lists for some crops. Driving this work at OECD level is however quite demanding for one single Member State. A range of analytical methods exists for dietary fiber and the best choice is still under debate. OECD has recently updated its lists of compounds for some crops. The EFSA GMO Panel expert welcomed this work since much knowledge has been gained in the past 15 years on plant metabolic interactions and it might be time to think about more targeted analysis focussing on pathways in the plants.

The Latvian delegate found a general analysis of impacts of consumption of a food (e.g. potential toxicity or allergenicity) more important than detailing single compounds analysis of a food.

Weight of evidence for assessment of interactions

Following a weight of evidence approach, taking account of the 4-stack, its parental stacks and individual single events, no indication of interaction that would impact on human and animal health was identified. The evidence included genetic stability, trait stability, protein expression level, known mode of action, agronomic and phenotypic stability, compositional analysis, nutritional assessment, and historical use of the crop. Against this background, comments were received on the type of toxicity studies and the need for animal feeding trials. The Hungarian delegate wanted to see

histological studies *in vitro* on human tissues as well as tests with whole food, not only with the microbially produced proteins. In response to the question whether this should not be dealt with in the assessment of the single events, the Hungarian delegate referred to lectins that are not fully degraded in the digestive system; she mentioned that there is a lack of evidence for absence of Cry receptors in the human gut since six Cry binding proteins are identified. She also referred to the finding of Cry1Ab in blood and umbilical cord - although no relation with GM food was demonstrated - and that a worker had developed allergy to Bt toxins in Bt sprays. A GMO Panel expert explained that binding of Cry proteins in intestinal tissue was not found in studies from Wageningen on lack of specific Cry binding to intestinal tissues[8]. The Dutch delegate recalled to concentrate on the safety assessment of interaction in this 4-stack and not on the assessment of single Cry protein binding *in vitro*.

The Austrian delegate stated that for stacks (as for all GM food) a repeated dose toxicity study should be performed while assessing interactions, and multigenerational toxicity studies and reproduction studies while assessing long-term effects. Hungary agreed with this approach.

In contrast, the Danish delegate reminded that when single events are approved, their combination in the food chain (i.e. in the kitchen) is also approved. To the Latvian delegate single compound testing through animal tests (as is typical for pharmaceutical testing) seemed too harsh for food safety testing. He underlined that European risk assessors should feel their responsibility for not giving false signals to other countries in the world; it would be better to focus on nutritional approaches instead of searching for unlikely unintended effects from mixing foods. A GMO Panel member mentioned the pressure for less animal trials on European level[9]. Alignment of policies across scientific fields is necessary when judging the need for animal tests for stacked GM events when there is no indication of interactions and when single events are approved.

Allergenicity

Allergenicity for workers was tested for this GM plant, although *in vitro* digestibility of the proteins, heat stability and bioinformatics indicated no changes in its allergenic property. No interactions were found in previous assessment studies that would have triggered more allergenicity studies.

The Norwegian delegate shared that the adjuvanticity of one of the Cry proteins is under closer study since 2004 and the Norwegian GMO Panel will comment on the report. The EFSA GMO Panel expert assured that this topic has been addressed during the work on the EFSA scientific opinion on allergenicity and in the context of GM plant applications; he also referred to the discussions between EFSA and the Norwegian GMO Panel regarding this topic.

The Hungarian delegate recalled that *in vitro* digestibility does not represent true digestion conditions, but did not suggest alternatives. The GMO Panel expert clarified that *in vitro* tests are part of the weight of evidence approach; taken alone they do not lead to risk assessment conclusions. Austria remarked that the Simulated Gastric Fluid test was done at pH1.2 instead of the recommended pH2.

Ireland asked to take a step back and to look at the bigger picture: since there are allergenic foods (peanuts) on the market, allergenicity is a risk management issue and the data provision in this GM case is sufficient. Small details under risk assessment cannot take away the existence of allergenicity.

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[8]Noteborn, H.P.J.M., Bienenmann-Ploum, M.E., van den Berg, J.H.J., Alink, G.M., Zolla, L., Reynaerts, A., Pensa, M. and Kuiper, H.A. (1995) Safety assessment of the Bacillus thuringiensis insecticidal crystal protein CRYIA(b) expressed in transgenic tomatoes. In: Genetically Modified Foods - Safety Aspects, ACS Symposium Series 605 (Engel, K.-H., Takeoka, G.R. and Teranishi, R., eds). Washington, DC: American Chemical Society, pp. 134-147. Chapter DOI: 10.1021/bk-1995-0605.ch012, http://pubs.acs.org/doi/abs/10.1021/bk-1995-0605.ch012

[9] MS activities in the area of alternatives for animal testing (the three Rs, i.e. reduction, replacement and refinement), is documented in the following report: ECOPA (2007) CONAM Survey – 3Rs Implementation in Europe, Report by CONAM Ethical Working group. European Consensus Platform for 3R-Alternatives, Brussels.
http://www.ecopa.eu/wp-content/uploads/CONAM_3Rs%20Implementation.pdf

9. PLENARY SESSION: FOLLOW-UP WITH THE NETWORK ON THE QUESTION TO SUBMIT MS DATA FOR THE EFSA PROCUREMENT CONTRACT FOR "ESTABLISHING A DATABASE OF BIO-ECOLOGICAL INFORMATION OF NON-TARGET ARTHROPOD SPECIES TO SUPPORT THE ENVIRONMENTAL RISK ASSESSMENT OF GENETICALLY MODIFIED CROPS IN THE EU"

Introduction

An EFSA GMO Unit scientist briefly introduced the EFSA project entitled "establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of GM crops in the EU".

The EFSA project has two objectives. The first objective is to establish a database that contains relevant information on non-target arthropod species. The second objective is to explore how the information contained in the database can inform the environmental risk assessment of GM crops; be it in terms of problem formulation, species selection, risk assessment studies or post-market environmental monitoring.

Following an EFSA open call for tenders, the contract was awarded to Jörg Romeis from Agroscope Reckenholz Switzerland (Contractor) who leads a team of experts that is composed of Fernando Alvarez-Alfageme and Michael Meissle (both from Agroscope Reckenholz) and that is supported by the subcontractor Louise Malone from the New Zealand Institute for Plant and Food Research. The project started in December 2010 and the final report is expected by June 2012. Different meetings between EFSA and the Contractor will take place to discuss progress and interim reports. The final outputs of the project will be made available to all on EFSA's website.

On 4 March 2011, EFSA sent a letter to the Competent Authorities under Directive 2001/18/EC and members of the GMO Network to inform EU Member States about the EFSA project. MS were invited to share relevant arthropod data from their region with the Contractor. As the Contractor and EFSA are also interested to consider the conservational status of arthropod species, lists of protected arthropod species or arthropod species of conservation concern pertaining to the EU are welcome.

Presentation by the Contractor

The Contractor, Jörg Romeis, presented the technical implementation strategy for the project; the experience gained with a previous EU maize fauna project; encountered problems and the expected outcomes of the project. He detailed the relevant information to populate the database, including taxonomy (including validation); geographical data (country; longitude/latitude coordinates); abundance (per species; sampling period; recorded taxonomic range); habitat (crop/field margin; primary habitat: below-ground, on the soil surface and above-ground plant parts; sampling method); ecological function including feeding guild and habits (primary functional group; adults and juveniles primary feeding guild); and references. The Contractor explained that such relevant information is being retrieved through systematic literature searches and he detailed the criteria for the selection of suitable publications and datasets; the crop species under consideration; the case studies; potential use of the database to support the environmental risk assessment of GM crops; and field margins. The Contractor indicated that grey literature (not archived and indexed in bibliographic databases), if accessible, may be used to complement the selection and screening of relevant publications.

Preliminary results reveal that available data on arthropod communities in European field margins are limited and diverse. Based on previous experience with the maize fauna database project (see Knecht

et al., 2010[10]), the Contractor reported that some countries, like Hungary, Germany and Czech Republic, have collected many arthropod data in maize fields, while few data are available for other (including maize growing) countries. Other challenges are that the majority of accessible studies only investigated a limited number of species, and did not necessarily provide a measure of species abundance, which is an important attribute to enter into the database. In some studies, arthropods were pooled into families or orders, and not identified to the species level.

Discussion

Members of the GMO Network expressed an interest to support the EFSA project by providing studies pertaining to their regions that contain relevant arthropod data collected in fields (including field margins) with maize, potato, sugar/fodder beet, oilseed rape, soybean, cotton and/or rice. All relevant information will be considered by the Contractor, irrespective of when the study was conducted or published or its language. However, to be useful, each study should contain information on: taxonomy, geography, abundance, habitat, and ecological function of non-target arthropods. Studies will be screened for their relevance for the completion of this project.

The importance of keeping the fauna database updated after the completion of the project was stressed by the Dutch delegate.

Upon a question from the Belgian delegate, it was confirmed that not only non-target arthropods will be included in the database, but also arthropod pests. Pests will be classified as herbivores.

The Polish delegate noted that the plant community composition in field margins may give an important indication on the possible occurrence of specific arthropods. The Contractor agreed that the plant community composition is likely more influencing arthropod assemblages in field margins than the neighbouring crop. Therefore, a better characterisation of plant communities in European field margins would be crucial to improve the quality and usefulness of field margin data in the database. This characterisation, however, cannot be achieved within the current project.

The UK delegate requested clarification on the role of the case studies. In line with the tender specifications, a number of case studies will be explored by the Contractor. A first set of case studies was defined in broad terms, and will include single insecticidal traits (such as lepidopteran- and coleopteran-active Bt-proteins); herbicide-tolerance traits; stacked traits (including multiple insecticidal traits and combinations of herbicide-tolerance and/or insect-resistance); and traits to nutritionally enhance crops. These case studies will serve as examples on how to make use of the database; they have an educational role, as they will be part of the report that will complement the fauna database. These cases will differ from each other in the specific risk hypotheses that have been identified and range from potential direct effects (e.g. potential toxicity of a certain Bt-protein to arthropods other than the target pest) to potential indirect effects (e.g. changes in the weed management practice related to the deployment of a herbicide-tolerance trait). It is also important to focus on the occurrence of unforeseeable unintended effects, which go beyond the intended effects of the transformation. Since no specific hypothesis can be formulated for these potential unanticipated unintended effects, a (protection goal) functional group-driven assessment is to be considered (e.g. based on the abundance of relevant functional groups).

EFSA indicated that the information contained in the fauna database does not relate to a specific stressor, meaning it could also support the environmental risk assessment of pesticides, and hence be used for other purposes (e.g. ecological modelling); it is the fact sheets and case studies that put the

[10] Knecht S, Romeis J, Malone LA, Candolfi MP, Garcia-Alonso M, Habustova O, Huesing JE, Kiss J, Nentwig W, Pons X, Rauschen S, Szénási A, Bigler F, 2010. A faunistic database as a tool for identification and selection of potential non-target arthropod species for regulatory risk assessment of GM maize. IOBC/wprs Bulletin 52, 65-69.

information contained in the fauna database in relation with the environmental risk assessment of GM crops.

Closing remarks

By bringing together and structuring relevant EU scientific literature and hence research results, the fauna database will provide baseline data on the geographical distribution and abundance of arthropods occurring in arable fields, as well as their ecological functions. It therefore enables to inform the problem formulation, the selection of test species for lower- and higher-tier risk assessment studies, or post-market environmental monitoring (e.g. case-specific monitoring).

10. ANY OTHER BUSINESS

Member States were invited to actively provide feedback through the online public consultation on the draft guidance on GM animals (http://www.efsa.europa.eu/en/calls/consultations.htm). The Network members were asked to notify their colleague experts on GM animals to provide comments on the FF aspects as well as the Animal Health and Welfare (H&W) aspects of this draft guidance.

A call for expressions of interest for Seconded National Experts (SNE) within EFSA is open until 3 November 2012 (http://www.efsa.europa.eu/en/vacancies/vacancy/sne101103.htm). Ms Olaru from Romania will join the EFSA GMO Unit to work on GM microorganisms and Molecular Characterisation during 2 years. Another SNE post is still vacant within the GMO Unit preferably to address ERA of GM plants. EFSA sees this as a great opportunity to work with MS experts.

The open call for EFSA Panel experts is open until 14 June 2011. The selection procedure will determine the composition of the Panels, including the GMO Panel, for the years mid 2012- mid 2015.

Furthermore, experts who want to support the EFSA Panels in a Working Group can sign-up any time in the expert database from the EFSA homepage. http://www.efsa.europa.eu/en/networks/expertdb.htm. The Network was asked to notify their experts.

EFSA also called for lead Member States volunteering to carry out the initial Environmental Risk Assessment of GM crops for cultivation as required by Regulation (EC) No 1829/2003. Also collaboration between two MS during this ERA would be accepted as more MS would gain experience in this important task.

11. CLOSING OF THE MEETING

In the spirit of enhancing communication amongst risk assessors of Europe, this meeting was designed to discuss relevant scientific issues among MS GM risk assessment experts. Topics where MS have a particularly relevant role, such as evaluation of new techniques, PMEM, Risk Assessment of applications and the collection of data on arthropods, were addressed. The scientific quality of the discussion in the GMO Network and the interest shown is a support for the EFSA GMO Panel and a support for risk assessment at European and National level. When building the agenda for the next meeting in the second quarter of 2012, feedback provided on this meeting will be taken into account.

The Chair thanked all participants for the constructive discussion and closed the meeting.

APPENDIX 1 - LIST OF PARTICIPANTS

Appointed experts of EU Member States and other EU countries

	LAST NAME	NAME	COUNTRY CODE	AREA OF EXPERTISE
1	Aasmo Finne	Merethe	NO	**ERA**
2	Arar	Chantal	FR	**FF**
3	Ball	Louise	UK	**ERA**
4	Bardócz	Zsuzsanna	HU	**ERA**
5	Batic	Martin	SI	**ERA**
6	Busuttil	Ingrid	MT	**FF**
7	Cuadrado	Carmen	ES	**FF**
8	Dabrowski	Zbigniew	PL	**ERA**
9	De Schrijver	Adinda	BE	**ERA**
10	Delledonne	Massimo	IT	**ERA**
11	Djilianov	Dimitar	BG	**ERA**
12	Drahovska	Hana	SI	**ERA**
13	Falk	Anders	SE	**FF**
14	Georgieva	Tzveta	BG	**FF**
15	Heissenberger	Andreas	AT	**ERA**
16	Jenes	Barnabas	HU	**FF**
17	Kjellsson	Gosta	DK	**ERA**
18	Kok	Esther	NL	**FF**
19	Mäe	Andres	EE	**ERA**
20	Meisner	Anke	DE	**FF**
21	Mikalsen	Arne	NO	**FF**
22	Muiznieks	Indrikis	LV	**FF**
23	Murray	Bernie	IE	**ERA**
24	Navratilova	Miloslava	CZ	**ERA**
25	O'Mahony	Patrick	IE	**FF**
26	Onori	Roberta	IT	**FF**
27	Ortego	Felix	ES	**ERA**
28	Ovesná	Jaroslava	CZ	**FF**
29	Pedersen	Jan W.	DK	**FF**
30	Regnault-Roger	Catherine	FR	**ERA**
31	Sarvas	Matti	FI	**ERA**
32	Sowa	Slawomir	PL	**FF**
33	Van der Wilk	Frank	NL	**ERA**
34	Welling	Annikki	FI	**FF**
35	Woegerbauer	Markus	AT	**FF**
36	Zupancic	Alenka	SI	**FF**
Apologies: Karavangeli Margarita (GR); Beatrix Tappeser (DE)				
Observers from of Candidate Countries				
1	Bucan	Ervin	YU	
2	Eser	Vehbi	TR	
3	Popovska	Suzana	MK	
4	Simic	Domagoj	HR	
Observers from the European Commission				
1	Kantorska	Kaja	DGSANCO	

Invited Experts			
1	Andersson	Christer	GMO Panel
2	Davies	Howard	GMO Panel
3	Jacobsen	Evert	Invited speaker (*Plant Breeding, Plant Sciences Group, Wageningen UR, the Netherlands*)
4	Kleter	Gijs	GMO Panel
5	Kuiper	Harry	GMO Panel
6	Romeis	Jörg	Invited speaker (*Agroscope Reckenholz-Tänikon Research Station ART, Zurich, Switzerland*)
7	Sweet	Jeremy	GMO Panel
EFSA GMO Unit			
1	Bergman	Per	
2	Devos	Yann	
3	Diveki	Zoltan	
4	Lheureux	Karine	
5	Liu	Yi	
6	Mestdagh	Sylvie	
7	Paoletti	Claudia	
8	Podevin	Nancy	
9	Rodighiero	Stefano	
10	Schoonjans	Reinhilde	
11	Waigmann	Elisabeth	

LIST OF ABBREVIATIONS USED

Bt : *Bacillus thuringiensis*
CSM : Case-Specific Monitoring
DUS : Distinctness Uniformity Stability
EC : European Commission
EFSA : European Food Safety Authority
ENV : Environment
ERA : Environmental Risk Assessment
ERA GD : Environmental Risk Assessment Guidance Document on GM plants for Applicants
EU : European Union
FF : Food and Feed
GM : Genetically Modified
GMO : Genetically Modified Organism
GS : General Surveillance
HT : Herbicide-Tolerant
NTO : Non-Target Organism
MC : Molecular Characterisation
MS : Member States
OECD : Organisation for Economic Co-operation and Development
PMEM : Post-Market Environmental Monitoring
RA : Risk Assessment
RM : Risk Management
WG : Working Group