Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
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O./ref.: WIV-ISP/41/BAC/2016_0683

Title: Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/DE/2011/99 from Syngenta under Regulation (EC) No. 1829/2003

Context

Application EFSA/GMO/DE/2011/99 was submitted by Syngenta on 7 July 2011 for the marketing of genetically modified (GM) maize Bt11 × 59122 × MIR604 × 1507 × GA21 for food and feed uses, import and processing, excluding cultivation within the European Union (EU), within the framework of Regulation (EC) No. 1829/2003[1].

The five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- Bt11 genetically modified with the *cry1Ab* and *pat* genes;
- 59122 genetically modified with the *cry34Ab1*, *cry35Ab1* and *pat* genes;
- MIR604 genetically modified with the *mcry3A* and *pmi* genes;
- 1507 genetically modified with the *cry1F* and *pat* genes;
- GA21 genetically modified with the *mepsps* gene.

It was therefore developed to achieve insect resistance (conferring protection against specific lepidopteran pests and coleopteran pests through expression of the Cry proteins) and herbicide tolerance to glyphosate-based (mEPSPS protein) and glufosinate ammonium-based (PAT protein) herbicides. It also expresses the PMI protein that was used as a selectable marker in maize MIR604.

The application was officially acknowledged by EFSA on 14 June 2012, which started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Seven experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually submitted to EFSA on 17 September 2012.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 15 July 2016 (EFSA Journal 2016;14(8):4567 [31 pp.].[2]), and published on 26 August 2016 together with the

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/4567

responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.
On 14 September 2016 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

It is important to note that the EFSA opinion on application EFSA/GMO/DE/2011/99 covers the five-event stack maize Bt11 × 59122 × MIR604 × 1507 × GA21 but also twenty subcombinations independently of their origin, resulting from the combination of any of the single events Bt11, 59122, MIR604, 1507 and GA21, and which were not previously assessed. Subcombinations occur as segregating progeny in the harvested grains of Bt11 × 59122 × MIR604 × 1507 × GA21, and refer also to any combination of up to four of the events Bt11, 59122, MIR604, 1507 or GA21 that has either been or could be produced by conventional crossing, through targeted breeding approaches. These are maize stacks that can be bred, produced and marketed independently of the five-event stack Bt11 × 59122 × MIR604 × 1507 × GA21.
Concerning these 20 subcombinations, the applicant provided no experimental data. The EFSA GMO Panel used a weight-of-evidence approach to conclude on the safety of these 20 subcombinations, which identified uncertainties due to data gaps. If these subcombinations were to be created in the future, the EFSA GMO Panel requests the applicant to provide information regarding expression levels of the newly expressed proteins, in order to reduce uncertainties.

In delivering the present advice the Biosafety Advisory Council considered in particular the information below:
- The comments formulated by the experts on application EFSA/GMO/DE/2011/99;
- The opinion of EFSA including the answers of the EFSA GMO Panel to these comments;
- The advices already adopted by the BAC on the five single events and five of the possible subcombinations. The conclusions of the BAC were as follows:

Event	Application number	BAC advice	Conclusions
GA21	EFSA/GMO/UK/2005/19 EFSA/GMO/RX-GA21	BAC/2007/SC614 (07/12/2007)	On the basis of the compositional analysis, the BAC agreed with the overall conclusion of the GMO panel of EFSA that: "it is unlikely that maize GA21 will have any adverse effects on human and animal health or on the environment in the context of its intended uses". The BAC was also of the view that EFSA should systematically request from the applicants the evaluation of the potential allergenicity of the whole GM plant or kernels, and that the power of the statistical analysis and/or the sensitivity of the tests performed on animals for toxicological and nutritional assessment need to comply with standards of good statistics in order to allow scientifically sound conclusions. Because of these remarks, some members of the BAC were not convinced that the health safety of this GM maize has been proven.
Bt11	EFSA/GMO/RX-Bt11	BAC/2009/0904 (17/03/2009)	No major risks for human and animal health or concerning the environment were identified.

MIR604	EFSA/GMO/UK/2005/11	BAC/2009/01365 (02/10/2009)	No major risks for animal health or concerning the environment were identified. The BAC disagreed with the GMO panel of EFSA that no risks for human health were identified, since identified potential allergenicity of the transgene protein (PMI) had not been tested *in vivo*. The BAC therefore gave a negative advice for the placing on the market of GM maize MIR604.
59122	EFSA/GMO/NL/2005/12 EFSA/GMO/RX-003 (currently open for MS consultation)	BAC_2007_SC_536 (14/06/2007)	No major risks for human and animal health or concerning the environment were identified.
1507	EFSA/GMO/NL/2004/02 EFSA/GMO/RX-1507	BAC/2009/1368 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified. At the time of the renewal, the lack of quality of animal trials for toxicity testing and testing of the nutritional value provided by the applicant, urged the BAC not to draw conclusions about the feed safety of this GM maize.
Bt11 × GA21	EFSA/GMO/UK/2007/49	BAC/2009/01493 (06/11/2009)	No major risks for human and animal health or concerning the environment were identified.
MIR604 × GA21	EFSA/GMO/UK/2007/48	BAC/2010/0952 (05/10/2010)	No major risks for animal health or concerning the environment were identified. A minority of the members of the BAC agreed with the GMO panel of EFSA that the maize MIR604 x GA21 was unlikely to have an adverse effect on human health in the context of its intended uses. A majority disagreed, since identified potential allergenicity of the transgene PMI protein had not been tested *in vitro* on serum of patients allergic to latex nor by appropriate *in vivo* tests. The BAC therefore could not give a univocal conclusive advice for the placing on the market of GM maize MIR604 x GA21.
Bt11 × MIR604	EFSA/GMO/UK/2007/50	BAC/2010/0956 (05/10/2010)	Same conclusion as for GM maize MIR604 x GA21.
Bt11 x GA21 × MIR604	EFSA/GMO/UK/2008/56	BAC/2010/0958 (05/10/2010)	Same conclusion as for GM maize MIR604 x GA21.
1507 x 59122	EFSA/GMO/NL/2005/15	BAC/2009/1366 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.

The ten GM maize mentioned in the table above are all authorised in the EU for food and feed uses[3].

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment[4].

2. Molecular characterisation

With regard to the molecular characterisation, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

Taking into account the previous assessment of the single events and the new data on compositional analysis provided by the applicant for the five-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize Bt11 × 59122 × MIR604 × 1507 × GA21, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, mEPSPS, PAT and PMI proteins in the context of previous applications, and no safety concerns were identified. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event should not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1Ab, Cry34Ab1, Cry35Ab1, mCry3A, Cry1F, mEPSPS, PAT and PMI proteins in the context of previous applications, and no concerns were identified. Since no new information on allergenicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid.
Previous advices of the Biosafety Council on GM maizes expressing the PMI protein (see applications EFSA/GMO/UK/2005/11, EFSA/GMO/UK/2007/48, EFSA/GMO/UK/2007/50, EFSA/GMO/UK/2008/56 and EFSA/GMO/DE/2010/82) reflected the concerns expressed by some of the members about the potential allergenicity of the PMI protein due to a possible cross-reactivity with a moderately important latex allergen, Hev b13. On the basis of recent updated expert opinions (see application EFSA/GMO/DE/2009/66), the Council is now of the opinion that further testing of the potential allergenicity of the PMI protein in humans is not needed from the safety viewpoint.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity.
With regard to the allergenicity of the whole GM plant, maize is not considered to be a common allergenic food. Based on the available information, the Biosafety Advisory Council

[4] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required according to EFSA procedure and was therefore not achieved.

considers that there is no evidence that the overall allergenicity of maize Bt11 × 59122 × MIR604 × 1507 × GA21 is changed as a result of the genetic modifications.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of maize Bt11 × 59122 × MIR604 × 1507 × GA21 - derived food and feed are not expected to differ from those of conventional maize varieties.

4. Monitoring

Since the allergenicity of the whole GM maize has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the advices already adopted by the BAC on the five single events and five of the possible subcombinations, and considering the data presently available, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that no major risks concerning the environment were identified;
2) Agrees with the GMO panel of EFSA that there is no reason to expect interactions between the newly expressed proteins that could impact on the food or feed safety;
3) Agrees with the GMO panel of EFSA that no major risks for animal and human health were identified;
4) Considers that the conclusions of the Biosafety Advisory Council on the five stacks that have been assessed previously (maizes Bt11 × GA21, MIR604 × GA21, Bt11 × MIR604, Bt11 x GA21 × MIR604, 1507 x 59122 – see table on pages 2-3 for further information) remain unchanged.

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Minority declaration
Annex II: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2011/99 and comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2012_0838)

Minority Declaration of P. Baret

In the present advice on application EFSA-GMO-DE-2011-99 (GM maize Bt11 × 59122 × MIR604 × 1507 × GA21), the assessment of the existing plant is extended to potential other combinations without any further testing or information on these potential combinations. It means that an assessment of genetically modified (GM) plants may be achieved without any data and based on assumptions on the extension of the collected evidence on some combination of events to other combinations of events.
Considering the minority opinion of Jean-Michel Wal in the ESFA opinion on the same dossier (see http://www.efsa.europa.eu/en/efsajournal/pub/4567) and the absence of formal elements on the potential combinations of GM construction, my opinion is that the advice should be restricted to existing combinations and not valid for potential combinations.

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Conseil de Biosécurité

17-09-2012

Secretariaat
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2011/99
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 15 June 2012
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Leo Fiems (ILVO), Rony Geers (KUL), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Bart Van Droogenbroeck (ILVO)
Domains of expertise of experts involved: Genetics, genome analysis, molecular characterisation, genetic engineering, transgene expression, human nutrition, analysis food/feed, substantial equivalence, animal nutrition, toxicology in vitro, general biochemistry, allergology, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/DE/2011/99** concerns an application of the company **Syngenta Crop Protection** for the marketing of the genetically modified **maize Bt11 x 59122 x MIR604 x 1507 x GA21** for food and feed applications under Regulation (EC) 1829/2003. The application has been officially acknowledged by EFSA on 14 June 2012.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5)

food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

Plants with multiple insertion events are likely to have more transformation-induced mutations and thus carry a greater risk of exhibiting unintended consequences. Genetically modified maize Bt11 x DAS-59122-7 x MIR604 x TC1507 x GA21 is obtained by traditional breeding of five genetically modified maize lines. So, the chance for mutations is limited.
Most individual events have been assessed and approved by EFSA (2005, 2007a,b, 2009a,b).
The applicant put forward that potential adverse effects to human and animal health arising from Cry1Ab, Cry34Ab1 and Cry35Ab1, mCry3A, Cry1F, PAT, MIR604 PMI and mEPSPS have previously been assessed and concludes that the potential toxic effects to humans and animals of these proteins could be considered negligible. Indeed, a series of test have been conducted, so that there a low risk for safety problems. However, the safety aspects are based on the safety aspects of the individual new inserted proteins. The safety aspects of the multiple challenge, due to the combination of the newly inserted proteins, are rather weakly demonstrated.
Some caution with regard to safety of maize Bt11 x DAS-59122-7 x MIR604 x TC1507 x GA21 is desirable.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

Clear introduction into the dossier, no comments.
-Typing error on pg. 21, second paragraph, last sentence “incluring” should be “including”
- Typing error pg. 21, last paragraph first sentence. The word “maize” is repeated at the start of the sentence.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No information missing, no comments. All provided info is clear.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No major comments. All the necessary information is provided.
Not that in this case the dossier for all single events combined in this stacked event have already been evaluated by EFSA and received positive scientific opinions. As a consequence no new information relevant to this section has been included in this application (this in contrast with the other dossier sent (GMO/DE/2010/86) for evaluation together with this application).

- One small remark, similar as in the other dossier: In Table C.2.1 (pg. 34) the applicant describes the origin of the 35S promoters used in the "active ingredient cassette" and the "selectable marker cassette, respectively.
For the 35S promotor in the "active ingredient cassette" with a size of 509 bp, the following description is given:
"Promoter from the cauliflower mosaic virus (CaMV) (Gardner et al., 1981), supplemented with the intron sequence 6 (471 bp) from the alcohol dehydrogenase 1S (adh1) gene from maize."
This could give the reader the idea that the intron sequence might be part of the promoter sequence. To avoid confusion it is better not to include the information of the intron sequence in the functional description of the promoter sequence. The intron itself is discussed as the second functional element in Table C.2.1 anyway. The same remark applies to the selectable marker cassette.
SBB: We agree with this comment, but as this is more a remark to the applicant (to adapt text), we wonder if it is relevant to send this remark to EFSA?
- In Table C.2.-2., (pg. 36), the RB sequence is included as an element of the active ingredient cassette, while in Table C.2.-3 (pg. 38). the RB sequence in included in the table as a vector backbone component (which seems more reasonable). Why is the RB of 59122 maize considered in another way as that of MIR604 maize?
SBB: This is because MIR604 and 59122 are from different companies.
In addition, Table C.2.-3. list all elements of the plasmid used, while table C.2.-2 on focuses on the T-DNA region. It would be better if all tables provide similar information (and e.g. also all use bp or kb).

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments or questions, as all traits and characteristics have already been evaluated by EFSA and received positive scientific opinions in the dossier of the 5 single events .

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No major comments or questions.
- Typing error, pg 50, in the description of the sequences inserted in 59122 'peroxidise' should be 'peroxidase'.
- I agree with the conclusions from Appendix 3, describing the Southern blot results of the stacked event in comparison with those of the single events:
" *In summary, the analyses showed that the predicted DNA hybridization patterns from each individual event were confirmed in Bt11 x 59122 x MIR604 x 1507 x GA21 maize demonstrating preservation of the integrity of the transgenic insert from each individual event to Bt11 x 59122 x MIR604 x 1507 x GA21 maize and therefore to all the sub-combinations of fewer of these events*"

- In addition, it is clear from the information derived from new BLASTN and BLASTX analyses that have been performed on the genomic sequences flanking the Bt11, 59122, MIR604, 1507 and GA21 maize inserts, using up-to-date nucleotide and protein databases, that the flanking regions are the same as in the single events and that none of the inserts in the stacked event disrupts known endogenous maize genes, as was the case in the single events.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

I agree with the conclusions from Appendix 10 regarding ELISA analysis of tthe expression of the proteins in stacked event, in comparison to the protein expression in the single events:
"These results confirm that, as expected, transgenic protein expression in Bt11 x 59122 x MIR604 x 1507 x GA21 maize is not substantially different from that of the Bt11, 59122, MIR604, 1507 and GA21 single maize events."

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

No comments. All provided information is clear.

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

No comments. All provided information is clear.
- From the conclusions formulated on pg. 54, based on the Southern blot analysis described in Appendix 3, I agree with the following statement about the stability of the inserts in the stacked event: "*The results obtained also confirm the genetic stability and absence of interactions between the inserts from Bt11, 59122, MIR604, 1507 and GA21 maize in the higher order stack maize."*
- In addition I agree with the statement formulated on pg. 80, which is based on ELISA expression study of the stacked and single events explained in detail in Appendix 10:
"*The results confirmed the phenotypic stability and that each of the traits derived from the single events are conserved in the stacked maize. The results also confirm that there is no evidence for any potential interactions or adverse effects arising from the combined expression of the introduced proteins in the higher order stack and therefore, there is no need for experimental data to be obtained for any of the sub-combinations."*

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

Maize Bt11 x 59122 x MIR604 x 1507 x GA21 is a combined trait obtained by conventional breeding. The single events have been previously approved. EFSA concluded that the single events are compositionally and agronomically equivalent to their conventional counterpart except for the presence of the intended traits.

The applicant states that there is no reason to expect that the combination by conventional breeding would result in changes in maize composition.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

The approach is in line with precious dossiers. A comparative study was conducted with maize Bt11 x 59122 x MIR604 x 1507 x GA21 and non-transgenic, near isogenic hybid maize grown at different locations. Commercial reference varieties were grown in adjacent plots of the eight locations.

The composition of maize Bt11 x 59122 x MIR604 x 1507 x GA21 was compared with the near-isogenic counterparts. The ILSI Crop Composition Database, 2010, was also used as a reference.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The approach is equivalent to previous dossiers.
I have no further comments on the selection of compounds for analysis of forage and grain.

The overall conclusion of the applicant is that maize Bt11 x 59122 x MIR604 x 1507 x GA21 is equivalent in composition to conventional maize, apart from the introduced traits.

I agree with this conclusion.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

In the previous dossiers a detailed description of the wet and dry milling technology was given. Taking into account the compositional equivalence, maize Bt11 x 59122 x MIR604 x 1507 x GA21 will be processed in the same way as conventional maize.

I agree with this conclusion.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

The values for maximum expression in kernels used in these calculations are all lower than the maximum values as shown in the 2009 US trial. Why weren't these values used?

SBB: The Technical dossier states: *"The dietary exposure assessment for Bt11 x 59122 x MIR604 x 1507 x GA21 maize is performed using the grain expression data of the proteins Cry1Ab, Cry34Ab1 and Cry35Ab1, mCry3A, Cry1F, PAT, MIR604 PMI and mEPSPS obtained in a study conducted in the*

US (Appendix 10 of this application – which contains the data of the 2009 US trial*). The maximum protein concentration for each of the newly expressed proteins was used to estimate potential exposure."*
In table D.7.7-2 the maximum protein concentration data have been included and they do correspond to the highest figures obtained during the 2009 US field trial, except for Cry3A where 0.29 is taken as the maximum concentration instead of 0.31.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

On P.101 of the Technical Dossier (7.8.1. Safety assessment of newly expressed proteins) the applicant stated that the potential adverse effects to humans and animals of the newly expressed proteins Cry1Ab, Cry34Ab1 and Cry35Ab1, mCry3A, Cry1F, PAT, MIR604 PMI and mEPSPS were previously evaluated as part of the risk assessments conducted to support the Bt11, 59122, MIR604, 1507 and GA21 import applications. This may not be a guarantee that the effect of a combination of events on toxicity is negligible, taking into account that the expression level of the introduced traits in the GM stacked event might be different from that of the GM parental lines (De Schrijver et al., 2007).
SBB: In this particular case, the expression levels of the single lines are comparable to the stacked event (see also comment of Van Droogenbroeck). Thus this reasoning is not applicable here...
A multi-generation study, performed with mice, showed that average litter size and weight, as well as number of weaned pups, were lower in NK603 x MON810 maize, compared to the near isogenic line, and differences were statistically significant in the 3rd and 4th litters (Velimirov et al., 2008).
SBB: We do not see why a multi-generation should be asked for. The double stacks and the triple Bt11xMIR604XGA21 have been considered safe. Considering the spectrum activity of the traits, there is no reason to believe that combining the traits will affect human/animal health.

Further, as far as we are aware the BAC has never raised the issue that multi-generation studies are necessary. Up till now, short-term feeding trials have been considered appropriate as a first step to assess potential adverse effects on animal health (before deciding if any further testing, e.g. multi-generation studies, is necessary.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

An additional 2 alignments to proteins from 2 species were identified as proteins described as "repressor proteins" however, these contain only motifs similar to EPSPS and a BLAST search with these proteins returns only EPSPS-similar proteins as the top alignments. The *E*-values for alignments between these sequences and the mEPSPS amino acid sequence ranged were 8.10 x 10^{-58} and 4.75 x 10^{-58}.
What exactly is meant by this?

SBB: We suppose this is a personal question and not a question to be posed to EFSA?

Reports concerning the updated assessment of amino acid sequence similarity between the transgenic proteins and known or putative toxins seem to be missing for Cry34Ab1, Cry35Ab1 and Cry1F.

SBB: The Technical Dossier states: "*Since 59122 and 1507 maize are not a Syngenta proprietary event, reference is made to technical data provided by Dow AgroSciences LLC to support the safety of 59122 and 1507 maize to human and animal health and the environment. In particular, bioinformatic analyses with known or putative toxins were carried out for 59122 and 1507 maize by Dow AgroSciences LLC for the assessment of the amino acid sequence similarity between the transgenic proteins Cry34Ab1, Cry35Ab1, Cry1F and PAT, as well as the translated reading frames with no minimum size at the genome to insert junctions (Table D.2(d)-1)*."

We consider the reference as sufficient.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a): 50-day feeding study in broiler chickens (Brake, 2011; app. 28)

Feed Consumption:

There was an overall difference in feed consumption due to maize grain source (0 to 48 days) where the broilers fed Bt11 x DAS-59122-7 x MIR604 x TC1507 x GA21 diets consumed less than the birds fed nontransgenic diets, and the birds fed NCSU 2009 diets were intermediate. The nontransgenic grain and diet exhibited the lowest moisture, which may have improved pellet quality that contributed to greater feed consumption. Males consumed significantly more feed than females at all time periods, as well as cumulatively (0 to 48), as expected due to their naturally larger size.

Further, there were no maize grain source-by-sex interactions for feed consumption.

What will be the effect of this on the outcome of the study?

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

It is assumed that it is unlikely that any interaction between the newly expressed proteins would alter the pattern of expression of endogenous proteins/potential allergens.

Comment 2

The potential for allergenicity of the individual traits has already been assessed in separate EFSA applications on the basis of an evaluation of the allergenicity of the source organisms, amino acid sequence comparisons, and the physicochemical properties and abundance of the individual proteins. This resulted in the conclusion of a low allergenicity risk for either of the individual traits. Combining these traits did not significantly alter the expression levels of the individual proteins except for the PAT protein where modestly increased expression levels were observed that however are unlikely to significantly affect uptake levels and hence allergenicity.

On p110, 2nd paragraph, the applicants indicate the likelihood that "*any interactions between the newly expressed proteins and metabolic pathways of maize would alter the pattern of expression of endogenous proteins/potential allergens*" are unlikely based on a conclusion reached by the EFSA GMO panel in reference EFSA, 2010d. I looked up this exhaustive document but did not find the conclusion referred to. I may have missed it and therefore would like a more precise reference for this strong statement.

SBB: The applicant is probably referring to the references EFSA, 2010 a, b or c (scientific opinions on AP 48, 50 or 56).

A final remark concerns enzymatic breakdown products from the targeted herbicides and pesticides. As I already mentioned in a previous report (report on dossier GMO/NL/2011/91), such breakdown products may accumulate in (parts of) the GM plant, cause toxicity upon consumption and/or modify endogenous proteins with increased risk for allergenicity. Yet, this feature is not addressed at all by the applicants although logic suggests an increased risk as a result of an increased resistance range of the GM plant.

SBB:The remark above relates to the still open discussion on where this kind of assessment should be made (pesticide legislation or GMO legislation).

Additional comment from SBB:

There are doubts regarding the potential allergenicity of PMI protein (Advice BAC on dossier EFSA/GMO/DE/2010/82). The applicant refers to sequence homology and the three-dimensional spatial structure of the PMI protein to conclude on absence of potential allergenic effect. The question remains : why has the applicant not performed in vitro and/or in vivo tests which could have taken away the doubts?

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

Bt11 x 59122 x MIR604 x 1507 x GA21 maize forage and grain may not be materially different in nutrient composition than forage and grain from the maize reference hybrids grown under the same conditions.
However, in genetic modified maize, in which the expression of Cry1Ab protein is introduced, principal component analysis showed metabolic variations involving 50% changes in osmolytes and branched amino acids (Manetti et al., 2006).
SBB: This reference is an article on MON810.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

This item is not relevant, as the application of maize Bt11 x DAS-59122-7 x MIR604 x TC1507 x GA21 is not intended for cultivation.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Because of some long-term effects in animals (see comments with regard to D.7.8 and D.9.7), it is questionable if Bt11 x 59122 x MIR604 x 1507 x GA21 maize will not exert a long-term effect in humans.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

A broiler experiment (Brake, 2011) showed no effects on animal performance, which was confirmed by Sauvé (2011). A flaw in the experimental design was not found.

Comment 2

From a 50-day broiler feeding study with Bt11 x 59122 x MIR604 x 1507 x GA21 maize grain it can be concluded that this genetically modified maize is safe for food and feed consumption. Furthermore, the combination of the introduced proteins within Bt11 x 59122 x MIR604 x 1507 x GA21 maize offers the

best chance of finding a potential adverse effect, because any adverse effect caused by combinations of some of the single maize events would also be apparent in the higher order stack.
However, short-term feeding experiments may not always be appropriate. Feeding MON810 maize, containing Cry1Ab protein, is safe for pigs, but there was a tendency for an increase in kidney weight (Walsh et al., 2012). Spiroux de Vendômois et al. (2009) detected GM-maize linked effects due to Cry1Ab either after 14 weeks of consumption or at a high GM feed dose in the diet. Blood cells, adrenal gland and kidney weights, blood urea nitrogen and spleen weight were affected. They observed a sex-dependency for the measured parameters in liver and kidneys.
SBB: The comment here relates to a broader discussion on whether short-term feeding trials are appropriate to assess potential adverse effects on animal health. As far as we are aware the BAC has never raised the issue that multi-generation studies are considered necessary. Up till now, short-term feeding trials have been considered appropriate as a first step to assess potential adverse effects on animal health (before deciding if any further testing, e.g. multi-generation studies, is necessary.
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D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

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