Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2018_0055

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2011-92 from Pioneer under Regulation (EC) No. 1829/2003

Context

Application EFSA-GMO-NL-2011-92 was submitted by Pioneer on 3 February 2011 for the marketing of genetically modified (GM) maize 1507 x 59122 x MON810 x NK603 and all its subcombinations 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 four-event stack maize 1507 x 59122 x MON810 x NK603 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- 1507, expressing the Cry1F protein for resistance to lepidopteran pests, and the PAT protein for tolerance to glufosinate ammonium-based herbicides;
- 59122, expressing the Cry34Ab1 and Cry35Ab1 proteins for resistance to lepidopteran pests and the PAT protein for tolerance to glufosinate ammonium-based herbicides;
- MON810, expressing the Cry1Ab protein for resistance to lepidopteran pests;
- NK603, expressing the CP4 EPSPS and its variant CP4 EPSPS L214P proteins for tolerance to glyphosate-containing herbicides.

The application was officially acknowledged by EFSA on 30 January 2012. At the same date EFSA 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. See Annex I for an overview of all the comments.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 14 November 2017 (EFSA Journal 2017;15(11):5000 [29 pp.][2]), and published on 28 November 2017 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

[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 https://www.efsa.europa.eu/en/efsajournal/pub/5000

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On 5 December 2017 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed.

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-NL-2011-92;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and five subcombinations (stacked events). The conclusions of the BAC for the most recent applications for the single events were as follows:

Event	Application number	BAC advice	Conclusions
1507	EFSA-GMO-RX-001	BAC/2017/0186 (21/03/2017)	No major risks for human and animal health or concerning the environment were identified. (minority declaration related to the lack of statistically convincing studies on toxicity)
59122	EFSA/GMO/RX-003	BAC/2017/0740 (19/09/2017)	No major risks for human and animal health or concerning the environment were identified.
MON810	EFSA/GMO/RX-MON810	BAC/2009_01510 (17/11/2009)	No major risks for human and animal health or concerning the environment were identified.
NK603	EFSA-GMO-NL-2005-22	BAC/2009/1367 (02/10/2009)	No major risks for human and animal health or concerning the environment were identified.

All GM maize mentioned in the table above are authorised in the EU for food and feed uses[3], as well as five combinations of two or more events.

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize 1507 x 59122 x MON810 x NK603 seeds (i.e. during transport and/or processing) into the European environment[4] will lead to any unwanted effects.

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, for the subcombinations previously assessed as well as for the subcombinations that were not previously assessed.

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 four-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize 1507 x 59122 x MON810 x NK603, in comparison with its conventional counterpart, do not raise safety concerns.

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm
[4] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, PAT and CP4 EPSPS 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 does not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, PAT and CP4 EPSPS 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.
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.

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 1507 x 59122 x MON810 x NK603-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 fully 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 four 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 the potential environmental release of maize 1507 x 59122 x MON810 x NK603 is unlikely to pose any threat to the European environment;
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 in the context of its proposed uses, maize 1507 x 59122 x MON810 x NK603 is unlikely to pose any risk to human and animal health;
4) Considers that the conclusions of the Biosafety Advisory Council on the single events that have been assessed previously (1507, 59122, MON810 and NK603 - see table on page 2) remain unchanged.

In addition the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM maize in the existing allergenicity monitoring systems.

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

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/92 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2012_0441)

Bioveiligheidsraad
Conseil de Biosécurité

26-04-2012

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2012_0441
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/92 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 10 Februari 2012
Coordinator: Prof. Dirk Reheul
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, plant biology, breeding techniques, human and animal nutrition, toxicology in vitro and in vivo, general biochemistry, allergology, insect resistance, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2011/92** concerns an application of the company **Pioneer** for the marketing of the genetically modified **maize 1507x59122xMON810xNK603** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 30 January 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. As the data for the single events have already been evaluated, the evaluation

of this maize with stacked events is focussed on the data related to the combination of transformation events. It was expected that the expert evaluated whether 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

1507x59122xMON810xNK603 maize was obtained by traditional breeding of single or double stacks GMO. The characterisation of all GMO (single or double stacks GMO) is well described on p. 19-22 of the application. Safety assessment have been realized for 1507 maize, 59122 maize and NK603 maize; MON810 has been authorized by EFSA for cultivation in EU.
For the risk management of GM stacked events from crosses between GM events, we refer to the article of De Schrijver et al. (2007). Until now no feed or food risk problems have been revealed for stacked events based on crosses from single stacks that were assessed to be safe.
However, potential risks can never be excluded, and therefor, a continious monitoring reveals necessary. We refer here to a recent paper (Hirshi, Trends in Plant Science, 2012, referred in cell-press) where it is stated that specific genetic material from plants (miRNA, fragments between 19-24 nucleotides attaching to mRNA hence inhibiting protein synthesis and thereby playing a central role in RNA-interference) can resist intestinal digestion, be absorbed and (in mice) influence gene expression.
Although this does not say anything about the DNA of GMO's, this new fact, if confirmed, will put a new light on the use of GMO's and the impact of such discovery should be integrated in an open discussion between all stakeholders in order to increase again a trust between science, industry, opinion makers and the broader public. If such information remains hidden or deliberately neglected it only can feed distract, and this is the last we want to happen.

Note SBB: We need to consider that people have been eating (non-GM) maize for quite some time (centuries) and that maize is considered as safe. The discovery that DNA/RNA can resist digestion, has not changed the notion that (non-GM) maize is safe for consumption.
Also important to consider is whether the transgenes present in the GM maize, might jeopardise its safe use and hence if monitoring is needed…

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

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

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

No questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

No questions

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/questions.

Comment 3

No questions

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

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

No questions

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

DNA insertions in the stacked events are similar as to the individual insertions in 1507, 59122, MON810 and NK603-maize.
No differences in expression of the proteins in the stacked versus the single events is concluded.
This is true for:
1507 x MON810
59122 x MON810
1507 x MON810 x NK603
But
No expression studies have been done for 59122 x MON810 x NK603, although all double combination have been done.

The “general” conclusion on p. 76 is therefor a bit too general as it is stated that protein expression levels are done in all possible substacks of these 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.

Comment 2

No comments/questions.

Comment 3

No questions

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

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

No questions

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

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No comments/questions.

Comment 3

No questions

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

There seem to be no significant differences between the event and its control concerning the antinutrient content.

Comment 2

As described in the initial letter the single events have already been evaluated. My comments will focus on the combination of transformation events.
The general approach is in line with previous dossiers: a comparison of maize 1705 x 59122 x MON810 x NK603 with a near-isoline comparator and other commercial maize hybrids.
No further questions.

Comment 3

I understand that the terminologies of “conventional herbicide-treated” or “untreated” (p. 97) alternate throughout the text and refer to conventional herbicide practices. However, I think it would be better to use just one terminology which is “conventional herbicide treatment” and which also refers better to the reality as done.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

The field study was conducted at six locations in the US including a randomized block design of maize 1705 x 59122 x MON810 x NK603 and the comparator. Two herbicide treatments were applied: a conventional herbicide treatment and a specific treatment with glyphosate and glufosinate.
The statistical models applied are widely accepted.

As it was the case in previous submissions the comparison included data obtained from field trials of commercial non genetically modified maize hybrids. Literature data on the nutrient composition were also used.
No further questions.

Comment 2

Comparisons were made of conventional herbicide-treated 4 stack event which near-isoline non-GM comparator (PHI-2008-091), obtained by crossing PH09 B x PH581.
Comparisons were made of intended herbicide-treated 4 stack event with near-isoline non GM-comparators.
Where there was a difference identified, this was compared to a corresponding tolerance interval, containing 99% of the values for corresponding analytes hybrids (4x2=8 different hybrids).

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Analysis of grain samples include proximate and fibre, fatty acids, amino acids, minerals, vitamins, secondary metabolites and anti-nutrients.

Relevant constituents are included. Some groups are analysed in detail such as vitamins, particularly tocopherols.

I regret to some extend that in such an in depth analysis no attention is given to carotenoids in maize. Only β-carotene is considered. No data are available for other important carotenoids in maize such as lutein, zeaxanthin and others. They are of growing importance in human nutrition.

Relevant secondary metabolites end anti-nutrients are included.

For most constituents no statistical differences were observed. This was however the case for some constituents but the values are within the tolerance interval.

Analysis of forage include proximate, fibre and minerals. The applicant concludes in the same direction as for grain.

As a conclusion for this item the applicant states that maize 1705 x 59122 x MON810 x NK603 is compositionally equivalent to non- GM control maize and commercial maize lines. This applies also to sub-combinations of the events.

I agree with this conclusion.

Comment 2

No questions

Additional comment SBB:
Analysis of carotenoids such as lutein, zeaxanthin and others is not foreseen in the maize OECD consensus document of 2002, and was therefore most likely not considered in the single events and the stacked events.
The expert didn't repeat his comment on the analysis of dietary fibres. The SBB confirms that in this application only results for ADF, NDF and crude fibres are given. For consistence with previous dossiers it is suggested to add the following comment:
"The compositional analysis should have included the analysis on dietary fibre, as this concept is widely accepted in human food studies".

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No questions

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

The applicant concludes that the specification of maize 1705 x 59122 x MON810 x NK603 is the same as that of conventional maize.
No further comment.

Comment 2

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No novel processing method is envisaged. Traditional well known methods will be used for the processing of maize 1705 x 59122 x MON810 x NK603. Wet milling and dry milling processes will be applied.

Maize is generally used for the production of products for human food, such as starch, syrups, maize oil and ethanol. In addition maize is used as an animal feed and for industrial products.
Due to the compositional equivalence, processing of maize 1705 x 59122 x MON810 x NK603 will be in the same way as for conventional maize.

The applicant demonstrated that the insert related proteins are easily denatured and degraded during conventional processing.

I agree with both statements.

Comment 2

No questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No particular questions.

Comment 2

No questions

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

According to the Technical Dossier Part 1:
The application seeks authorisation for the placing on the market of GM 1507x59122xMON810xNK603 maize for import, processing and all food and feed uses in accordance with Art.3(1) and 15(1) of regulation (EC) 182912003. IN ADDITION the application also seeks authorisation of the sub-combination of events, independently of their origin.
GM events intended to commercialise:

- 1507xMON810
- 1507xMON810xNK603
- 1507x59122xMON810

This is in agreement with the EFSA-Guidance EFSA Journal (2007) 512, 1-5.

The consequences of the combined use of the herbicides on the stacked events:

Data is lacking concerning the occurrence, levels and fate of residues of the herbicides and their metabolites in the plant tissues and the potential adverse health effects as indirect effects associated with their use on human and animal health. Although the effect of the herbicides on human and animal health falls under Directive 91/414/EC, it is the duty and responsibility of the toxicologist assessing the risk of the genetic modification to evaluate and discuss the complete picture of the genetic modification. As the herbicides are used as integral parts of the biotechnology-based weed management strategy, the risk assessment must also consider their potential impact on human and animal health.
GM 1507x59122xMON810xNK603 maize is developed to be able to use a herbicide regime with both glyphosate and glufosinate. Data concerning the use of the herbicides in the field trials is available. However, no data is made available concerning the identification and quantification of the herbicides and metabolite residues in the GM plants and grain used for food/feed. As the use of the herbicides is linked to the genetic modification, the applicant should make the residue data available and make an estimation of the anticipated intake (food/feed).

Note SBB: As said this falls under another regulation and any comments made on this issue will not be treated by EFSA for this particular reason. For application GMO/UK/2005/20 (maize 59122xNK603) a similar question raised by one of the experts has not been transmitted to EFSA.

Comment 2

Table 31 gives the TMDI (theoretical maximum daily intake) for the different proteins, and table 9 (p. 136-137) gives the toxicological evaluation of the new proteins (by EFSA), but what about the doses of combined Cry-proteins in the stacked events ?
Even if there are no interactions between the inserts (cfr. p. 139-140) the cumulative Cry-protein levels should be considered.
In case of acute toxicity studies in rats, should not the combined proteins (Cry1F, Cry34Ab$_1$,Cry35Ab, Cry1Ab) be used ? and doses of each of them adapted for the combination study ?

Note SBB: Comparison of expression levels between quadruple stack, and single events is present in Table 10 & 11. No statistically significant differences were found (see also p.73-74).

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

No new genetic modifications have been introduced in maize 1507x59122xMON810xNK603.
The safety of the proteins CRY1F, CRY34Ab1, CRY35Ab1, CRY1Ab, PAT, CP4 EPSPS has been confirmed in detail in accordance with the applications of authorisation of maize 1507 (and renewal), maize 59122, maize MON810 (and renewal), and maize NK603 (and renewal). Maize 1507x59122xMON810xNK603 was obtained by traditional crossing of the 4 GM single parental maize events. The inserts were all integrated into different loci in the maize nuclear genome. Updated bioinformatics evaluations were provided.

Further Comments:
What about combined toxicity? No repeated dose 28-day oral toxicity study in rats is provided which includes test groups with a mixture of the microbial-produced CRY1F, CRY34Ab1, CRY35Ab1, CRY1Ab, PAT, CP4 EPSPS proteins in the diet (3 doses). In addition, no reference can be made to sub-chronic feeding studies: No 90-day feeding studies in rodents are made available for maize 1507x59122xMON810xNK603 or the stacked events intended to be commercialised.

No further questions.

Note SBB: According to the updated EFSA guidelines on food/feed, toxicity studies are not a standard requirement for GM stacked event.
EFSA states *"In the case of GM plants containing stacked events, toxicological testing of the whole food and/or feed derived from the GM plant should be considered when there are indications of possible interactions between the events stacked within the GM plant. Such indications may be obtained from the outcome of the molecular characterization, and knowledge of the mode of action of the newly expressed proteins, and possibly from the compositional characterization of the GM plant containing stacked events."*

Comment 2

All proteins have been tested earlier. No acute toxicity was found.

Comment 3

No further questions.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comments or questions.

Comment 2

No further questions.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Compositional analysis of 1507x59122xMON810xNK603 maize grain and forage (Annex 6c/d: Califf and Maxwell, 2009).

The nutrient compositional analysis was performed on grain and forage of a field study with maize grown in one growing season (2008) in the USA at only six separate locations (Annex 6: Hettinger et al., 2010). According to the EFSA Guidance (EFSA Journal 2011; 9(5):2150) each field trial should be replicated at a minimum of eight sites!

Groups used in the statistic analysis: 1) Near-isoline PH09BxPH581 F1 hybrid: conventional herbicide-treated; 2) 1507x59122xMON810xNK603 maize: conventional herbicide-treated; 3) 1507x59122xMON810xNK603 maize: glyphosate/glufosinate treated.

Grain:

Fatty Acids: The across sites analysis revealed statistically different **oleic acid** (C18:1)↑ and **linoleic acid** (C18:2)↓ concentrations between 1507x59122xMON810xNK603 maize treated with gly/glu or conventional herbicide treated and the non-GM comparator.

Minerals: The across sites analysis revealed a statistically significant increase in the **potassium** content of 1507x59122xMON810xNK603 maize treated with gly/glu or conventional herbicide treated compared to the non-GM comparator.

Forage: ok

Taking also the outcome of the compositional analysis of previous assessed stacks (maize 1507x59122; maize 59122x1507xNK603; maize NK603xMON810; maize 1507xNK603) into account: no consistent trends were observed.

Furthermore, the observed changes did not have an impact on the nutritional properties of GM-maize derived feed as tested in the 42-day poultry study (Annex 16: Smith, 2009). However, this study could not be fully assessed because only the study report was provided, the raw data and statistic analysis were not provided.

Note SBB: In the first package that the expert received Annex 16b with raw data was missing. This document was sent later to the expert.

Comment 2

No further questions.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

According to the Technical Dossier Part 1:

The application seeks authorisation for the placing on the market of GM 1507x 59122xMON810xNK603 maize for import, processing and all food and feed uses in accordance with Art.3(1) and 15(1) of regulation (EC) 182912003. IN ADDITION the application also seeks authorisation of the sub-combination of events, independently of their origin. The inserts were al integrated into different loci in the maize nuclear genome.

The applicant submitted a nutritional performance study, but no 90-day feeding toxicity study in rodents.

42-day poultry feeding study (Annex 16: Smith, 2009).
Groups: 1) Non transgenic near-isogenic control maize 091 grain: conventional herbicide-treated; 2) 1507x59122xMON810xNK603 maize grain: conventional herbicide-treated; 3) 1507x59122xMON810xNK603 maize grain: glyphosate/glufosinate treated, and 3 reference maize grains 4) 33H25, 5) 33M15, 6) 33D11.
According to the authors no adverse effects could be detected. However, no proper review could be made of this study. Only the Annex16a file is made available (the study report). No raw data/ statistic analysis was provided (Annex16b).

Long-term impact on human and animal health
Several applications concerning stacked transformation events including MON810, NK603, 1507 and 59122 have been assessed by EFSA.
Adopted applications:

Application	Single or Stacked event	90-day rat feeding study	Broiler study
UK-2005-21	59122x1507xNK603	No study	♀: kidney weight
NL-2005-15	1507x59122	♀: ↑ RBC counts, hematocrit, ♀: ↓ MCH, serum chloride, serum sodium	No valid study
UK-2004-05	1507xNK603	No study	OK
UK-2005-20	59122xNK603	No study	Reference was made to 59122x1507xNK603
UK-2004-01	NK603xMON810	No study	OK
RX-NK603 and NL-2005-22	NK603	♂, ♀: ↓ RBC, ↑ platelets, ↑ haemaglobin, ↑ hematocrit, ♂: ↑ liver weight, ↑ heart weight (kidney weight not included in the study)	♂, ♀combined: breast meat weight (kg), fat pad weight (kg, % of live weight, thigh meat moisture (%)
NL-2005-12	59122	haematology ♂: ↑ MCH, ↑ MCHC, ↓ RDW, ↓ ARET ♀: ↑ platelets ♀: ↑ uterus weight ♂: glandular dilatation in the stomach	♀: ↑ liver weight
RX-MON810	MON810	♀: ↑ platelets ♀: ↓ MCHC ♀, ♂: ↓ albumin/globulin ratio	OK
RX-1507 and NL-2004-02	1507	♂, ♀: ↓ RBC, ↓ platelets, ↓ haemaglobin, ↓ hematocrit, ♂: ↓ kidney weight	OK

No medium-term feeding studies are made available for the stacked events intended to be commercialised.

In addition, only one 90-day feeding study in rats is available from a former submitted and adopted application: the stacked event 1507x59122 maize. This 90-day rodent feeding study was performed in order to investigate the likelihood of possible interactions between the 3 Cry proteins Cry34Ab1, Cry35Ab1, Cry1F (Appenzeller et al., 2009). However, what are the synergism and combinatorial, cumulative effects of the expression of 4 Cry proteins (Cry1Ab, Cry34Ab1, Cry35Ab1, Cry1F), the expression of the proteins PAT and CP4 EPSPS and the combined use of the glyphosate and glufosinate herbicides targeting amino acid metabolism, on human and animal health? What is the long-term impact of combined exposure to glyphosate and glufosinate ammonium and metabolites, breakdown products in the GM plant material? No medium-term rodent toxicity feeding study is made available. In the nutritional performance study submitted for the application of the stacked event maize 59122x1507xNK603 (EFSA/GMO/UK/2005/21) stat. sign. differences were observed in relative kidney weight in female rats.

Note SBB: SBB: Potential interactions have been evaluated: see p.139-142. It is concluded that any interactions – if they would occur – would not affect food/feed safety.

For application EFSA/GMO/UK/2005/21 (maize 59122x1507xNK603) no question/comment was raised by our experts regarding differences in kidney weight by female rats. This is also not mentionned in the advice of the Council for this application.

Further in the answers to comments raised by the member states EFSA answers :"The Panel is of the opinion that since 59122x1507xNK603 maize is compositionally and agronomically equivalent to conventional maize and the possibility of interactions between the expressed proteins was not identified, no toxicological or nutritional feeding studies are required to conclude on the safety of 59122x1507xNK603 maize and considers that the feeding study provided further confirms this conclusion."

Recently scientific groups reviewed the effects of GM crops on mammalian health based on reports and publications made available for the adopted applications (Spiroux de Vendômois et al., 2009; Séralini et al., 2011; Dona and Arvanitoyannis, 2009; Domingo and Bordonaba, 2011). In addition, the biological effects of the stacked event maize NK603xMON810 was studied in long-term reproduction studies in mice (Velimirov et al, 2008): the fertility of mice fed NK603xMON810 maize was found to be impaired with fewer offspring being produced than by mice fed control maize.

In conclusion:
Taking all available data into consideration, unintended direct or indirect effects cannot be excluded without performing toxicity testing in rodents. At least a medium-term 90-day feeding study in rodents should be performed according to the principles of OECD guideline 408 following an adapted protocol according to the EFSA Guidance (EFSA –Q-2009-00941).

Note SBB: In the food/feed guidance of EFSA the following statement is made: "If the composition of the food and/or feed derived from GM plant is substantially modified, or if there are any indications for the potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses, not only new constituents but also the whole food and feed derived from the GM plant should be tested. In such case the testing program should include a 90-day toxicity study in rodents ».

According to expert's comments under sections 7.1 to 7.3, the substantial equivalence between this GM maize and non GM control has been demonstrated.

Also the molecular data and phenotypic analysis do not give indications for unintended effects.
Further the 42-day broiler studies did not observe significant differences with test diets.

Comment 2

a) 42-day feeding study in broiler chickens (Smith, 2009 (annex 16 PHI-2008-258)).
Performance

Growth performance

No statistically significant differences were observed between broilers consuming 091 control and 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603+Gly/Glu test diets.
In addition, all observed values of growth performance measures for broilers fed 091, 1507x59122xMON810xNK603, and 1507x59122xMON810xNK603+Gly/Glu test diets fell within the tolerance intervals calculated for this study.

Organ and carcass yield

No statistically significant differences in either kidney or liver yields were observed between broilers consuming 091 control and 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603+Gly/Glu test diets.
All observed kidney and liver yield values for broilers fed 091, 1507x59122xMON810xNK603, and 1507x59122xMON810xNK603+Gly/Glu test diets fell within the tolerance intervals calculated for this study.

No statistically significant differences were observed for carcass or individual parts yields between 091 control and 1507x59122xMON810xNK603 or 1507x59122xMON810xNK603+Gly/Glu test diet groups. All observed yield values were within the tolerance interval calculated for this study.

b) 90-Day rat feeding study ()).

Not performed. No further testing is needed.

Comment 3

No further questions.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

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. However, combining these traits in hybrid plants may affect allergenicity by (i) altering the expression levels of the individual proteins and/or (ii) by generating novel metabolites with allergenic potential though enzymatic interactions between the stacked proteins.

Expression levels of the transgene-encoded proteins were comparable to those in the parental lines and conservative estimates of dietary exposure show low to very low exposure levels. Therefore, the main risk for increased allergenicity derives from the potential for novel interactions between the stacked proteins and/or their enzymatic by-products. Toxicology data on the (unaltered) composition of four-trait maize grain and forage and on the (high) substrate specificity of the individual enzymes, along with the natural occurrence of certain enzyme combinations in non-allergenic microbial organisms and the assessment by EFSA of specific sub-stack combinations do not indicate an increased risk for allergenicity as a result of unintended interactions between the different enzymes introduced. The conclusion of the applicant that the 1507x59122xMON810xNK603 maize does not pose an increased risk for allergenicity therefore is justified.

Comment 2

No further questions.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No further questions.

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)

Comment 1

The mode of action of Cry-proteins as well as of PAT (glufosinate) and CP4-EPSPS (glyphosate-tolerance) is well explained.

However, it is stated on p. 155 that the stack event 1507x59122xMON810xNK603 maize expresses the bacterial form of the EPSPS-enzyme with a lower affinity for glyphosate, and furthermore it is concluded for a glyphosate insensitivity of the stacked event !!
Is it a lower affinity ? or an insensitivity for glyphosate ? of the enzyme and hence of the stacked event?

Note SBB: Due to the lower affinity/sensitivity of the CP4 EPSPS (which is the bacterial EPSPS introduced in the GM plant) to glyphosate, GM maize obtains his tolerance to glyphosate.

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)

Comment 1

No questions

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions

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

See earlier remarks on the totality of the Cry-proteins (see D.7.8).

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

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)

Comment 1

No questions

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No questions

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

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

Comments/Questions of the expert(s)

Comment 1

See earlier remarks in general information about the possibility of fragments between 14-24 nucleotides resisting intestinal digestion in mammals, being absorbed an influencing gene expression.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

References

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