Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2011-94 from Bayer CropScience under Regulation (EC) No. 1829/2003

Adopted on 29 May 2018
Ref. SC/1510/BAC/2018_0326

Context

Application EFSA-GMO-NL-2011-94 was submitted by Bayer CropScience on 18 February 2011 for the marketing of genetically modified (GM) cotton GHB614 x LLCotton25 x MON15985 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 three-event stack cotton GHB614 x LLCotton25 x MON15985 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- GHB614, expressing the 2mEPSPS protein for tolerance to glyphosate-containing herbicides;
- LLCotton25, expressing the PAT protein for tolerance to glufosinate ammonium-based herbicides;
- MON15985, expressing the Cry1Ac and Cry2Ab2 proteins for resistance to certain lepidopteran pests.

The application was officially acknowledged by EFSA on 15 July 2015. 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 Service Biosafety and Biotechnology (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 7 March 2018 (EFSA Journal 2018;16(4):5213[2]), and published on 20 April 2018 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/fr/efsajournal/pub/5213

Biosafety Advisory Council - Secretariat • Service Biosafety and Biotechnology (SBB)
Sciensano • Rue Juliette Wytsmanstraat 14 • B-1050 Brussels • Belgium
T + 32 2 642 52 93 • bac@sciensano.be • www.bio-council.be

In delivering the present advice the Biosafety Advisory Council considered in particular the folowing information:
- The comments formulated by the experts on application EFSA-GMO-NL-2011-94;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events and one subcombination (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
GHB614	EFSA-GMO-NL-2008-51	BAC/2009_924 (21/04/2009)	No major risks for human and animal health or concerning the environment were identified.
LLCotton25	EFSA-GMO-NL-2005-13	BAC/2007_SC_461 (12/03/2007)	No major risks for human and animal health or concerning the environment were identified.
MON15985	EFSA-GMO-UK-2008-57 and RX-MON15985	BAC/2014_0733 (28/10/2014)	No major risks for human and animal health or concerning the environment were identified.

All GM cottons 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 cotton GHB614 x LLCotton25 x MON15985 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.

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 three-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM cotton GHB614 x LLCotton25 x MON15985, 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 2mEPSPS, PAT, Cry1Ac and Cry2Ab2 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] 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.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed 2mEPSPS, PAT, Cry1Ac and Cry2Ab2 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 cotton GHB614 x LLCotton25 x MON15985-derived food and feed are not expected to differ from those of conventional maize varieties.

4. Monitoring

Since the allergenicity of the whole GM cotton 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 three single events and one subcombination, and considering the data presently available, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of cotton GHB614 x LLCotton25 x MON15985 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, cotton GHB614 x LLCotton25 x MON15985 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 (GHB614, LLCotton25 and MON15985 - see table on page 2) remain unchanged.

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

Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/94 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2015_0708)

Bioveiligheidsraad
Conseil de Biosécurité

20/10/2015

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2015_0708
Email. : bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/94
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 3 August 2015.
Coordinator: Philippe Baret
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Jan Van Doorsselaere (KATO).
Domains of expertise of experts involved: Molecular characterisation, DNA/RNA/protein analysis, herbicide tolerance, animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens, breeding techniques, plant biology.
SBB: Didier Breyer, Fanny Coppens, Martine Goossens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2011/94** concerns an application submitted by **Bayer CropScience AG** for authorisation to place on the market genetically modified cotton **GHB614 x LLCotton25 x MON15985** in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.

The application has been officially acknowledged by EFSA on 21st July 2015.

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/questions received from the experts

GENERAL COMMENTS

Comment 1
There is no strict reason to prohibit the import of GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 and their use for food and feed purposes in the EU.
EFSA has concluded that cotton GHB614, LLCotton25 (EFSA, 2014a) and cotton MON15985 (EFSA, 2014b) are unlikely to have adverse effects on human and animal health and the environment, in the context of their intended use. So, it would be illogical to formulate constraints with regard to GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton.
However there may be some concern about the safe use of glyphosate and glufosinate ammonium in genetically modified herbicide tolerant crops, such as GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton.

Comment SBB
The assessment of potential risks associated with the use of herbicides is not within the remits of the BAC.

Comment 2
No comment.

Comment 3
GHB614 expresses EPSPS, giving tolerance to glyphosate due to the modified 5-enolpyruvyl-shikimate-3-phosphate synthase.
LLCotton25 expresses PAT (phosphinothricin acetyl-transferase) that acetylates glufosinate ammonium and thereby inactivates the herbicide.
MON15985 expresses Cry1Ac and Cry2Ab2 proteins that confer resistance to lepidopteran insects by interacting with different specific receptor sites in the target insects.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

A.1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) THE PARENTAL PLANT

Comment 1
No comment, adequate information is provided.

Comment 2
No questions.

Comment 3
No comment.

A.2. MOLECULAR CHARACTERISATION

A.2.1. INFORMATION RELATING TO THE GENETIC MODIFICATION including:

- Description of the methods used for the genetic modification
- Source and characterization of nucleic acid used for transformation
- Nature and source of vector(s) used

Comment 1

No comment, adequate information is provided.

Comment 2

No comment.

A.2.2. INFORMATION RELATING TO THE GM PLANT including:

- Description of the trait(s) and characteristics which have been introduced or modified
- Information on the sequences actually inserted or deleted
- Information on the expression of the insert
- Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

Comment 1

No comment, adequate information is provided.

Comment 2

GTxLLxB2 by conventional crossing of GHB614, LLCotton25 and MON15985.

Comment 3

No comment.

GM cotton containing events GHB614, LLCotton25 and MON15985 originated due to conventional crossing. The separate events have been approved by EFSA. The inserts are stable and expression of the inserts is comparable with the parental lines.

Comment SBB

The Biosafety Council also issued positive advices on the three single events, respectively on 21/04/2009 for cotton GHB614 (ref. BAC_2009_924), on 12/03/2007 for LLCotton25 (ref. BAC_2007_SC_461) and on 28/10/2014 for cotton MON15985 (ref. BAC_2014_0733).

A.3. COMPARATIVE ASSESSMENT

A.3.1. CRITERIA FOR THE SELECTION OF COMPARATOR(S)

Comment 1

Parental lines GHB614, LLCotton25 and MON15985, and the non GM counterpart variety FM958 were used, while the parental lines also used FM958 for introgressing the genetic modification.

Comment 2

Cotton GHB614 x LLCotton25 x MON15985 will be further referred in this evaluation as cotton 2015.
Cotton 2015 has been obtained by traditional breeding of lines containing single events, that have been evaluated before. No new genetic modification is applied.
It is unlikely that cotton derived by crossing of approved lines will result in a cotton with a different nutritionally composition.
A comparative assessment was performed in order to demonstrate the compositional and nutritional equivalence between cotton 2015 and the non GM comparator FiberMax 958, with a comparable genetic background and the three parental lines.
Cotton grain was collected from field trials at seven locations in the US.
No remarks on the field trials and the statistical analysis applied.
No remarks on this overall approach.

A.3.2. FIELD TRIALS: EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Comment 1

The six treatments, taking into account the plant variety descriptors with or without glufosinate and/or glyphosate, were appropriate; no further questions.

Comment 2

No particular remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1

The results of the compositional analysis showed a compositional and nutritional equivalence for most of the components assessed. However, significant differences were noticed for some compounds. It is somewhat amazing that differences between the GM cottonseeds and the non-GM counterpart amounted to approximately 20% for calcium, alpha tocopherol and total gossypol, which may be the result of the experimental design, due to diverging soil types, history of the parcels, ... Nevertheless, all values were within the reference ranges for commercial cotton seeds, so that differences are not really relevant from a food and/or feed safety perspective.
Statistically significant differences were also mentioned for phytic acid (p.78, p.79 and p.85 in the Technical dossier). However, the mean values were not shown in the tables, nor were they presented in the text. Reference ranges of phytic acid for commercial cotton seeds were neither mentioned. Phosphorus is not fully digestible for monogastric animals, when dietary phosphorus is bound to phytic acid, and phosphorus is one of the most important minerals in animal nutrition. So, a higher phytic acid content in GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 may require extra dietary phytase to cleave and to free the bound phosphates.
Fortunately, cottonseed meal is not frequently used in diets for pigs and poultry (Boucqué and Fiems, 1988).

Comment SBB

Data related to the analysis of phytic acid are indeed not provided in the technical dossier (table 16). They are however given in the detailed results (see p. 16, table 2.6.5 of appendix “Oberdorfer R 2010a.pdf”). The data are as follows:

Compound	Non-Transgenic FM958 Conventionally treated	Transgenic GTxLLxB2 Conventionally Treated	Transgenic GTxLLxB2 Treated with treated Test Herbicides	Reference Range
	Mean ± SD	Mean ± SD	Mean ± SD	
Phytic acid (% dm)	1.77 ± 0.23	1.60 + 0.20	1.63 + 0.23	0.85-2.57

The SBB has drawn the attention of the expert on these data. On this basis the expert is of the opinion that "the phytic acid content in the DM of GHB614 x LLCotton25 x MON15985 cotton is within the reference range. Phytic acid is an antinutrient; so the lower concentration in the GM cotton is rather a positive aspect. Consequently, the differences between non-GM and GM cotton do not represent a safety concern." Furthermore, the expert does not believe that extra clarification on this point is necessary.

Comment 2

- What is the meaning of the sentence on p.75 of technical dossier 2nd paragraph: "in all other cases substantial equivalence could not be excluded"?
- Why is cyclopropenoid fatty acid lower in the transgenic GHB614 compared to the non-transgenic control? This is not found anymore when GTxLLxB2 is compared to the non-GM counterpart (for cyclopropenoid fatty acid)

Comment sent to EFSA (text from the coordinator)

Considering that some differences in composition were significant between the GM cotton and its non-GM counterpart, the substantial equivalence cannot be demonstrated and a full toxicology testing should have been asked for.

Comment 3

Despite the fact that some significant differences in anti-nutrients were measured between the stacked line and the reference line on the one hand and the parental lines on the other hand, the values are within the reference range from literature.

Comment 4

The compounds were selected according to the OECD guidelines:

- proximates: protein, fat, ash, carbohydrates and moisture; results are given for carbohydrates as such and for acid detergent and neutral detergent fibre,
- aminoacids: the analysis covers the whole range of amino acids including the indispensible amino acids,
- fatty acids: results are given for the range of fatty acids from C14 to C24 including saturates, mono-unsaturates and poly-unsaturates,
- vitamins: results are limited to α-tocopherol; no other tocopherols, tocotrienols or other vitamins are included
- minerals: calcium phosphorous, magnesium, potassium, iron, zinc,
- anti-nutrients: gossypol is given as free and total gossypol, cyclopropenoid acids as malvalic, sterculic and dihydrosterculic acid; all known anti-nutrients in cotton are included

If statistically significant differences are found, results are discussed in terms of natural variations, biological and nutritional relevance.

It is demonstrated that grain from cotton 2015 is compositionaly and nutritionally equivalent to grain from the non GM comparator and the parental lines. Spraying with herbicides has no effect on the composition.
Taking into account the results presented, I agree with this overall conclusion.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment
No questions.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions.

Comment 2
As the same processing methods will be used for cotton 2015 as for the non GM comparator and the parental lines, no particular effects have to be expected.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment
The protein expression levels of 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS and NptII measured in leaves, squares and seed plant tissues of GTxLLxB2 are comparable with the levels observed in GHB614, LLCotton25 and MON15985 parental lines.

A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

- Molecular and biochemical characterisation of the newly expressed proteins
- Up-to-date bioinformatic search for homology
- Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM plant
- Data concerning the resistance of the newly expressed protein to proteolytic enzymes
- Repeated dose toxicity studies using laboratory animals

Comment 1
Based on the weight of evidence in this dossier it is unlikely that GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton will pose serious risks for toxicity. Single events GHB614, LLCotton25 (EFSA, 2014a) and MON15985 (EFSA, 2014b) are as safe as their conventional counterparts and non-GM reference varieties with respect to potential effects on human and animal health and the environment. Because there is no biological pathway in which the newly-inserted genes would directly or indirectly interact, there is no plausible or testable hypothesis for the interaction of the new proteins in GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton (Steiner et al., 2013).

A side effect of the use of GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton may be an increased use of herbicides in genetically modified herbicide tolerant crops. Herbicide use in the USA on soybean, corn and cotton declined slightly in the first years following introduction of herbicide resistant GM crops, but increased moderately in recent years (Fernandez-Cornejo et al., 2014), whereas Benbrook (2012) reported that herbicide-resistant crop technology has led to a 239 million kg increase in herbicide use in the USA between 1996 and 2011.
The continued application of the same herbicides in subsequent rotations may lead to increased selection pressure for herbicide resistant weed populations. Furthermore, the continued application of the same herbicides may result in an increased accumulation of residues in plant tissues (Bøhn et al., 2014; Rubio et al., 2014). Health concerns with regard to the use of glyphosate and glufosinate, and their metabolites have been reported recently: Garry et al., 2002; Gasnier et al., 2009; George et al., 2010; Carman et al., 2013; Samsel en Seneff, 2013; Zouaoui et al., 2013; Guilherme et al., 2014; Krüger et al., 2014; Laugeray et al., 2014; Mesnage et al., 2014; Ackermann et al., 2015; Guyton et al., 2015; Seneff et al., 2015. Food and feed that compromises human and animal health is unacceptable. Therefore, the application doses of the herbicides in weed management should be rigorously respected. These considerations emphasize the importance of an appropriate weed management. Herbicide mixing of glyphosate and glufosinate exposes weeds to multiple mechanisms of action, which may delay resistance evolution, at least temporarily. But using herbicide mixes may increase the quantity of herbicidal compounds required. Herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run (Evans et al., 2015).
GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton are not intended for cultivation in Europe, but there may be some concern about the safe use of glufosinate ammonium in GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton, and the import of GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton products to Europe for food and feed purposes.

Comment sent to EFSA (text from the coordinator)
The continued application of the same herbicides may result in an increased accumulation of residues in plant tissues (Bøhn et al., 2014; Rubio et al., 2014) with possible impact on animal and human health. How are these aspects taken into account in the dossier?

Comment 2
No new genetic modification was introduced in the stacked events, so no further questions about safety of those proteins of the single events (2mEPSPS, PAT, Cry2Ab2).

Comment 3
For the proteins 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, GUS and NptII earlier studies have demonstrated:
- Rapid degradation in SGF
- Rapid degradation in SIF (Cry2Ab2 digested to a stable core)
- No acute toxicity

Homology search: these studies are not up-to-date (2010). Please provide more recent studies.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment
Not applicable.

A.4.4. ASSESSMENT OF ALTERED LEVELS OF FOOD AND FEED CONSTITUENTS

Comment
Not relevant.

A.4.5. ASSESSMENT OF THE WHOLE FOOD AND/OR FEED DERIVED FROM GM PLANTS

Comment 1
No questions about safety for whole GM food/feed.
- Why were the data of the broiler chicken feeding study not shown?
- It is stated that the feeding study was performed with male broiler chickens (Stafford, 2010), but in the next paragraph a study is mentioned on male and female poultry for evaluating the effects of a feed component over an entire life span and under conditions of rapid growth: is this a different study?
Again, nothing is shown, why??

Comment SBB
The data of the broiler chicken feeding study are indeed not provided in the technical dossier, but a reference is made to the scientific appendix "Stafford, 2010" where the detailed results can be found (see also comment below). This study clearly mentions that male and female broiler chickens have been used.

Comment 2
a) 42-day poultry feeding study (Stafford, J., 2010)
Following 42 days of daily exposure to GlyTol x BGII x LL25 toasted cottonseed meal (dietary content of approximately 10%), there were no adverse effects detected in survival, measured body weights, body weight gain, feed consumption, feed conversion ratio, or weight of chilled carcass, legs, thighs, wings or breasts of ROSS#308 broiler chickens, when compared to exposure to non-GM counterpart cottonseed meal. Chickens consuming a diet containing 10% GlyTol x BGII x LL25 cottonseed meal demonstrated health and growth characteristics comparable to chickens consuming non-GM counterpart or commercial cottonseed meal diets.

b) 90-day rat feeding study (author).
Not performed. No further testing is needed.

Comment sent to EFSA (text from the coordinator)
No data are provided on a 90-day rat feeding study. They should have been provided as there is no substantial equivalence based on the compositional analysis (see previous comment).

A.5. ALLERGENICITY ASSESSMENT

A.5.1. ASSESSMENT OF ALLERGENICITY OF THE NEWLY EXPRESSED PROTEIN including:

- Amino acid sequence homology comparison between the newly expressed protein and known allergens using a comprehensive database
- Specific serum screening
- Pepsin resistance and in vitro digestibility tests
- Additional tests

Comment 1

Based on the weight of evidence in this dossier it is assumed that GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton do not pose a serious allergenic risk, and that they are comparable with conventional cotton with regard to allergenicity.

Comment 2

No comment, adequate information is provided.

Comment 3

No questions.

Comment 4

As indicated by the applicant, no new genetic modification was introduced in the combined cotton event GTxLLxB2 and the subcombination LLxB2. This reflects the GMO having been obtained by conventional crossing between three genetically modified cotton parental lines: GHB614, LLCotton25 and MON15985. The individual parental GMOs have been previously evaluated by EFSA (EFSA Opinion, 2006; EFSA Opinion, 2009; and EFSA Opinion, 2014), concluding the absence of an allergenic potential of the proteins 2mEPSPS, PAT, Cry1Ac, Cry2Ab2, NPTII and GUS expressed in the parents GHB614, LLCotton25 and MON15985. Finally, the absence of water-soluble allergens in cottonseed oil, the main food product for human use, is correlated with no clinical allergy observations after consumption of cottonseed oil. Therefore, no allergic reactions are anticipated from the current use pattern in the case of GHB614 x LLCotton25 x MON15985.

Specifically regarding the newly expressed proteins in GTxLLxB2 (and LLxB2), the applicant considered a further assessment of allergenicity as unnecessary (p. 107 of the technical dossier). This was based on the absence of newly expressed proteins in GTxLLxB2 (besides the proteins inherited from the parental GMO's) and on previous evaluations by EFSA concluding a lack of allergenic potential of the newly expressed proteins in the parental cotton GMOs. **I do not agree with this proposition and in fact consider the dossier as incomplete with regards to the allergenicity assessment of the newly expressed proteins.** The dossiers submitted before to EFSA for a risk assessment of the parental GMOs date from 2005 to 2008. This means that the present dossier bases itself on an antiquated bio-informatics analysis of the '*Amino acid sequence homology comparison between the newly expressed protein and known allergens using a comprehensive database*'. An updated analysis is thus required and is lacking in this dossier. The expert is aware that in other EFSA dossiers an updated analysis of (some of) the proteins of interest has been reported. However, this expert is of the opinion that when assessing an individual dossier, the expert needs to rely on the information provided in the dossier itself (and on public knowledge), but not on information provided in confidential dossiers by other applicants. Therefore, an updated analysis of the amino acid sequence homology with known allergens (and toxins) using recent databases is needed in this dossier and

should be provided by the applicant. As a consequence, it is not possible to fully assess the allergenicity of the newly expressed proteins in the GTxLLxB2 and LLxB2 GMO.

Comment SBB
This dossier has been submitted in 2011. It is common practice for EFSA to request from the applicant updated bio-informatic analyses at the latest just before the finalisation of its opinion.

A.5.2. ASSESSMENT OF ALLERGENICITY OF THE WHOLE GM PLANT

Comment 1
No questions.

Comment 2
See my comments above in the first paragraph.
Notwithstanding the assessment of the newly expressed proteins, the likelihood that the allergenic potential of the whole plant has increased is indeed very low.

A.5.3. ADJUVANTICITY

Comment 1
Not addressed here.

Comment 2
No increased risk.

A.6. NUTRITIONAL ASSESSMENT

A.6.1. NUTRITIONAL ASSESSMENT OF FOOD DERIVED FROM GM PLANTS

Comment 1
There is no reason to assume that the genetic modification has affected the nutritional value of food derived from GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton.

Comment 2
No questions.

A.6.2. NUTRITIONAL ASSESSMENT OF FEED DERIVED FROM GM PLANTS

Comment 1
There is no reason to assume that the genetic modification has affected the nutritional value of feed derived from GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton. Using MON15985 cotton in lactating Holstein cows, Castillo et al. (2004) reported a similar dry matter intake, milk yield, milk composition, body weight, and body condition when single event MON 15985 cotton,

which is part of the stacked events GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985, was compared to non-transgenic control and reference cottonseed.

Comment 2
No questions.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment
Anticipated intake of GHB614 x LLCotton25 x MON15985 and LLCotton25 x MON15985 cotton is not extensively described. The anticipated intake is not elaborated for monogastric and ruminant animals.

C. RISK CHARACTERISATION

Comment
No questions.

D. POST MARKET MONITORING (PMM) OF FOOD AND FEED DERIVED FROM GM PLANTS

Comment
p. 113: food produced from GTxLLxB2 cotton “does not present ethical or religious concerns…..”. I would suggest to omit this part of the sentence since it is a conclusion that the different stakeholders in the debate may reach, but should not be an *a priori* statement from the applicant.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment
No questions.

E.2. GENERAL APPROACH OF THE ERA

-

E.3. SPECIFIC AREAS OF RISK

As stated in the EFSA guidance on the environmental risk assessment of genetically modified plants (EFSA Journal 2010, 8(11):1879) the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. For each specific risk the ERA consists of the six steps described in Directive 2001/18/EC:
1. Problem formulation including hazard identification,
2. Hazard characterisation,

3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

E.3.1. PERSISTENCE AND INVASIVENESS INCLUDING PLANT-TO-PLANT GENE FLOW

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable in view of the non-included authorization for cultivation of GTxLLxB2 and LLxB2 cotton seeds in the EU.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable.

E.3.3. INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable.

E.3.4. INTERACTION BETWEEN THE GM PLANT AND NON-TARGET ORGANISMS (NTOS)

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable.

E.3.5. IMPACTS OF SPECIFIC CULTIVATION AND MANAGEMENT AND HARVESTING TECHNIQUES

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable.

E.3.6. Effects on biogeochemical processes

Comment 1
No comment, adequate information is provided.

Comment 2
Not applicable.

E.3.7. Effects on human and animal health

Comment 1
No new food or feed safety concerns are expected when stacked transgenes are not expressed in the same tissues or when their products are not translocated to the same tissues (Steiner et al., 2013).

Comment 2
No questions.

E.3.8. Overall risk evaluation and conclusions

Comment
No questions.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment
No questions.

E.4.2. Case-specific GM plant monitoring

Comment
No questions.

E.4.3. General surveillance for unanticipated adverse effects

Comment
No questions.

E.4.4. REPORTING THE RESULTS OF MONITORING

Comment
No questions.

References

Ackermann, W., Coenen, M., Schrödl, W., Shehata, A.A., Krüger, M. 2015. The influence of glyphosate on the microbiota and production of botulinum neurotoxin during ruminal fermentation. Curr. Microbiol. 70, 374-382.

Benbrook, C.M. 2012. Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Env. Sci. Eur. 24, Article 24, 13 pp.

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, R. 2014. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chem. 153, 207-215.

Boucqué, C.V., Fiems, L.O. 1988. Vegetable by-products of agro-industrial origin. Livest. Prod. Sci. 19, 97-135.

Carman, J.A., Vlieger, H.R., Ver Steeg, L.J., Sneller, V.E., Robinson, G.W., Clinch-Jones, C.A., Haynes, J.I., Edwards, J.W. 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. J. Org. Syst. 8, 38-54.

Castillo, A.R., Gallardo, M.R., Maciel, M., Giordano, J.M., Conti, G.A., Gaggiotti, M.C., Quaino, O., Gianni, C., Hartnel, G.F. 2004. Effects of feeding rations with genetically modified whole cottonseed to lactating Holstein cows. J. Dairy Sci. 87, 1778-1785.

EFSA, 2014a. Scientific Opinion on application (EFSA-GMO-NL-2010-77) for the placing on the market of herbicide-tolerant genetically modified cotton GHB614 LLCotton25 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 12, 3680, 26 pp.

EFSA, 2014b. Scientific Opinion on applications (EFSA-GMO-UK-2008-57 and EFSAGMO-RX-MON15985) for the placing on the market of insect-resistant genetically modified cotton MON 15985 for food and feed uses, import and processing1 , and for the renewal of authorisation of existing products produced from cotton MON 159852 , both under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 12, 3770, 42 pp.

Evans, J.A., Tranel, P.J., Hager, A.G., Schutte, B., Wu, C., Chatham, L.A., S Davis, A. 2015. Managing the evolution of herbicide resistance. Pest Manag Sci. 71, in press, doi: 10.1002/ps.4009.

Fernandez-Cornejo, J., Wechsler, S., Livingston, M., Mitchell, L., 2014. Genetically Engineered Crops in the United States, Economic Research Report 162, USDA, Washington DC, USA. 54 pp.

Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E., Burroughs, B.L. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River valley of Minnesota, USA. Environ. Health Perspect. 110, Suppl 3, 441-449.

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184-191.

George, J., Prasad, S., Mahmood, Z., Shukla, Y. 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach. J. Proteomics 73, 951-964.

Guilherme, S., Santos, M.A., Gaivão, I., Pacheco, M. 2014. DNA and chromosomal damage induced in fish (Anguilla anguilla L.) by aminomethylphosphonic acid (AMPA) - the major environmental breakdown product of glyphosate. Environ. Sci. Pollut. Res. 21, 8730-8739.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490-491.

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.W., Lutz, W., Shehata, A.A. 2014. Detection of glyphosate residues in animals and humans. J. Environ. Anal. Toxicol. 4, Article 210, 5 pp.

Laugeray, A., Herzine, A., Perche, O., Hébert, B., Aguillon-Naury, M., Richard, O., Menuet, A., Mazaud-Guittot, S., Lesné, L., Briault, S., Jegou, B., Pichon, J., Montécot-Dubourg, C., Mortaud, S. 2014. Pre- and postnatal exposure to low dose glufosinate ammonium induces autism-like phenotypes in mice. Front. Behav. Neurosci. 8, Article 390 (14 pp).

Mesnage, R., Defarge, N., Spiroux de Vendômois, J., Séralini, G.E. 2014. Major pesticides are more toxic to human cells than their declared active principles. BioMed Res. Int. Article ID 179691, 8 pp.

Rubio, F., Guo, E., Kamp, L. 2014. Survey of glyphosate residues in honey, corn and soy products. J Environ. Anal. Toxicol. 4: 249. doi: 10.4172/2161- 0525.1000249, 8 pp.

Samsel, A., Seneff, S. 2013. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. Entropy 15, 1416-1463.

Seneff, S., Swanson, N., Li, C. 2015. Aluminum and glyphosate can synergistically induce pineal gland pathology: Connection to gut dysbiosis and neurological disease. Agric. Sci. 6, 42-70.

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., Hannah, L.C. 2013. Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiol. 161, 1587-1594.

Zouaoui, K., Dulaurent, S., Gaulier, J.M., Moesch, C., Lachâtre, G. 2013. Determination of glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication. Forensic Sci. Int. 226, e20-e25.