Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2014-122 (genetically modified cotton GHB614 x T304-40 x GHB119) from Bayer CropScience N.V. under Regulation (EC) No. 1829/2003

11 September 2018
Ref. SC/1510/BAC/2018_0703

Context

Application EFSA-GMO-NL-2014-122 was submitted by Bayer CropScience N.V. for the marketing of genetically modified (GM) cotton GHB614 x T304-40 x GHB119 (Unique Identifier BCS-GH002-5 x BCS-GH004-7 x BCS-GH005-8), for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

The three-event stack cotton GHB614 x T304-40 x GHB119 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- GHB614, expressing the 2mEPSPS protein that confers tolerance to herbicide products containing glyphosate;
- T304-40, expressing Cry1Ab protein that confers resistance to certain lepidopteran pests, and the PAT protein that confers tolerance to herbicide products containing glufosinate ammonium;
- GHB119, expressing Cry2Ae protein that confers resistance to certain lepidopteran pests, and the PAT protein that confers tolerance to herbicide products containing glufosinate ammonium.

The application was validated by EFSA on 30 April 2015. A formal three-month consultation period of the Member States was started, lasting until 21 February 2017, 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). Nine 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 and the comments forwarded to EFSA.

The opinion of the EFSA Scientific Panel on GMOs was published on 25 July 2018 (EFSA Journal 2018;16(7):5349[2]). The responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period were published on 10 August 2018. On 13 August 2018 these two documents were forwarded to the Belgian experts. They were invited to give comments and to react if needed.

[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://doi.org/10.2903/j.efsa.2018.5349

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 BAC considered in particular the following information:
- The comments formulated by the experts on application EFSA-GMO-NL-2014-122;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single 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.
T304-40	EFSA-GMO-NL-2011-97	BAC/2014/0141 (14/03/2014)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
GHB119	EFSA-GMO-UK-2010-96	BAC/2016/0789 (19/12/2016)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.

All GM cotton events mentioned in the table above are authorised in the EU for food and feed uses[3].

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 T304-40 x GHB119 (i.e. during transport and/or processing) into the European environment[4] will lead to environmental harm.

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 T304-40 x GHB119, 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, Cry1Ab and Cry2Ae 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 cultivation of the GM crop in the EU, a full environmental assessment is as in the case of a cultivation file is not warranted.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed 2mEPSPS, PAT, Cry1Ab and Cry2Ae 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 T304-40 x GHB119-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 whole set of data on cotton GHB614 x T304-40 x GHB119 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, the answers of the EFSA GMO panel to the questions raised by the Belgian experts, and the advices already adopted by the BAC on the three single events, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of cotton GHB614 x T304-40 x GHB119 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 T304-40 x GHB119 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, T304-40 and GHB119 - 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-2014-122 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2017_0010)

Bioveiligheidsraad
Conseil de Biosécurité

06/01/2017

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2017_0010
Email. : bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2014/122
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 25 October 2016.
Coordinator: Philippe Baret
Experts: Eddy Decuypere (KUL), Patrick du Jardin (ULg-Gembloux), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB-UGent), Jan Van Doorsselaere (KATO), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, DNA/RNA/protein analysis, animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens, agronomy, breeding techniques, plant biology.
SBB: Didier Breyer, Fanny Coppens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2014/122** concerns an application submitted by the company **Bayer** for authorisation to place on the market genetically modified **cotton GHB614 x T304-40 x GHB119** 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 16 February 2016.

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
The risk for human and animal health and the environment of using (import & processing) the pyramided GHB614 x T304-40 x GHB119 cotton is considered negligible, because no new proteins were inserted compared to each of the single event cotton lines.
However, the increased use of glyphosate-tolerent and glufosinate-tolerent genetically modified crops and the resistance to Bt toxins highlight the potential vulnerability of Bt crops. Therefore, extra attention should be paid to the sustainability of the use of GHB614 x T304-40 x GHB119 cotton.

SBB comment: The assessment of pesticide use is not within the remit of the BAC.

Comment 2
This GM-cotton GHB614xT304-40xGHB119 is a stacked event obtained by conventional crossing and expressing 2mEPSPS protein conferring tolerance to glyphosate (from GHB614), PAT/bar protein conferring tolerance to glufosinate (from T304-40 and GHB119), and Cry1Ab (from T304-40) and Cry2Ae (GHB119) proteins conferring resistance to certain lepidopteran pests.

Comment 3
GHB614 x T304-40 x GHB119 cotton is obtained by crossing single parental lines using traditional breeding techniques. No new genetic modification was introduced in GHB614 x T304-40 x GHB119 cotton.

Comment 4
No comment.

Comment 5
Considering the assessment of stacks when all single events have been previously assessed, possible interactions between the combined events and their expression products need to be evaluated. The rationale of the applicant (following the reference Steiner et al 2013 quoted in the Technical dossier) is fine and the conclusions of the applicant are justified.
NB: I found no specific place in this form for concluding on this topic, hence I put it here.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
Cyclopropenoid fatty acids are responsible for giving a positive Halphen test; it is based on what? How can it be used to characterise cottonseed oil (for almost 100 years)? Characterize on what?

No further questions.

Comment 2
No comment.

Comment 3
None

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 questions;
No indications of potential interactions between the single events or between the newly expressed proteins.

Comment 2
No comments.

Comment 3
No comment.

Comment 4
None

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 questions.

Comment 2
No comments.

Comment 3
No comment.

Comment 4

1- Most of the updated bioinformatic analyses date from 2014. Is this recent enough? I would say yes, but I wonder which is the formal requirement of the implementing regulation and efsa's recent guidelines (if any).
(**SBB Comment**: Regulation (EU) No 503/2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 states under Annex II, II. SCIENTIFIC REQUIREMENTS, 1.2.2.2.(e): "Bioinformatic analyses shall be conducted

using up-to-date databases", without further precision. However EFSA usually requests an update of the bioinfo analyses from the applicant, before adoption of its opinion.)

2- When the applicant performed the bioinformatic analysis of the putative ORF translation products of event T404-40, one hit was found between the allergen database and the product of an ORF (identity over 8 AA with soybean allergens, technical dossier page 48 and ref M-411811-03-1-2 page 8). The assessment of the applicant is the following: "*The results of the 8-mer homology search showed identity between the T304-40_ORF.100 and two putative allergens from soybean. However, this identity is likely to occur by chance and not to represent an actual IgE epitope. Therefore, the putative gene product of T304-40_ORF.100 is not likely to possess allergenic properties. »*

The fact that the identity 'likely occurred by chance' seems a poor justification of safety. The applicant should be asked to perform a convincing risk assessment of this identity and possibly new hazard. To address this issue, the applicant could perform e.g. hydropathy plot analysis / 3D-modeling of the allergenic proteins (epitopes are expected at the hydrophilic surface of the protein). In line with efsa's guidelines, where no bioinformatic arguments are enough for concluding on safety, expression analysis of this ORF should be conducted.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
No questions

Comment 2
GHB614 x T304-40 x GHB119 cotton was compared with to its conventional counterpart as well to other non-GMO reference varieties.

Comment 3
None

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

Comment 1
The selected sites are sufficiently divergent to conduct a comparative analysis under different agro-environmental conditions, and all groups (GHB614xT304-40xGHB119 with conventional herbicide management and intended herbicides, conventional counterpart and additional comparators with conventional herbicides), were included at all sites.

Comment 2
Eight field trials were organized in field locations representative for cotton production in the USA.

Comment 3
Not evaluated.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1

Although some compounds (protein, fat, ash, carbohydrates, ...) of GHB614 x T304-40 x GHB119 cotton are significantly different from conventional cotton, differences are not relevant, because the values are within ranges reported in the literature.

Comment 2
The values of dihydrosterculic acid were always lower for GHB614xT304-40xGHB119 cotton compared with conventional counterpart as well as with reference varieties. Since this is one of the antinutrients in cottonseed that is lower in the GMO, it is no problem as for the equivalence between GHB614xT304-40xGHB119 and the conventional counterpart and reference varieties from a food and feed safety and nutritional point of view.

However, from a scientific point of view any possible explanation or hypothesis should be welcome; this is not given at any place in the report!!

Comment 3
Each field trial included a comparison of GHB614 x T304-40 x GHB119 cotton treated with intended herbicides, GHB614 x T304-40 x GHB119 cotton with conventional herbicides and the conventional counterpart cotton with conventional herbicides. Six non-GMO reference cotton varieties with conventional herbicide treatment were also included.

Constituents for comparative analysis were selected according to the OECD guidelines:
- proximates: moisture, crude protein, crude fat, ash, carbohydrates by difference
"carbohydrates by difference" is only acceptable for comparative purposes like in this study; there are methods for the direct assessment of the different carbohydrate classes; this modern approach gives much more information on the composition of the carbohydrate fraction; this is important in actual food policy issues,
- fibre: acid detergent and neutral detergent fibre
similar observation as for carbohydrates: only acceptable for comparative purposes
- minerals: calcium, potassium, phosphorus, magnesium, sodium, iron, copper, manganese, zinc
no remarks
- tocopherols: total tocopherols, α, β, γ and ó tocopherol are determined
good approach with respect to anti- oxidative vitamins, but no information on tocotrienols and no other fat soluble vitamins included
- anti-nutrients: known constituents with anti-nutrient properties in cottonseed are assessed: free en total gossypol, phytic acid, cyclopropenic acids: malvalic, sterculic and dihydrosterculic acid,
- amino acids: the whole range of amino acids has been studied,
- fatty acids: information on the whole range of fatty acids is available

As a conclusion on the selection of compounds it can be said that the approach according to the OECD guidelines is adequate for comparative purposes but not for up to date nutritional information.

No differences were observed, during the statistical evaluation, between GHB614 X T304-40 X GHB119 cotton with different herbicide treatment and conventional cotton, for most parameters. In some cases the proof of equivalence showed equivalence more likely, equivalence less likely than not and even non equivalence.

The applicant demonstrated that the magnitude of the reported levels lack biological relevance from a food and feed and nutritional point of view.

I agree with this conclusion

As an overall summary the applicant concluded that the statistical analysis supports the conclusion of equivalence in composition between GHB614 X T304-40 X GHB119 cotton and the reference varieties.

Taking into account the natural variations no biological relevant differences and/or lack of equivalence between GHB614 X T304-40 X GHB119 cotton and its comparator were observed.

I agree with this conclusion.

Comment 4
There seem to be no problems with the anti-nutrients content.

Comment 5
Not evaluated.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
No questions.

Comment 2
No remarks.

Comment 3
Although statistically significant difference in the trait 'lint percentage' with non-GM counterpart and non-equivalence with conventional cotton variety comparators were shown (see technical dossier page 78), this agronomic trait raises no safety concern within the scope of this application.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions

Comment 2
The applicant emphasizes that GHB614 X T304-40 X GHB119 cotton is not different from conventional cotton with the exception of the expressed proteins. No particular effects are expected. The particular proteins are denatured during processing as they are sensitive to forces applied. They are losing their functional activity.
The applicant concludes that it is highly unlikely that the derived food en feed products of GHB614 X T304-40 X GHB119 cotton will be different from conventional cotton.
I agree with this conclusion.

Comment 3
Not evaluated

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1

No indications of any toxicological effects for any of the newly expressed proteins, not with toxicity studies, nor with up-to-date bioinformatics search for homology with any toxic protein.
Importantly, no indications for any interactions between the newly expressed proteins, as they all are expressed in the same plant and seeds.
It is clearly shown that the 2mEPSPS, PAT/bar, Cry1Ab and Cry2Ae proteins have each very specific activities, with different substrates and different pathways in cotton. Moreover, in view of the absence (or near-absence) of changes in composition (endogenous components) in GMO cotton, it is unlikely that interactions between these new proteins and metabolic pathways of cotton would alter the pattern of expression of endogenous proteins, toxins or anti-nutrients.
The slight but significant decrease in dihydrosterculic acid in GHB614xT304-40xGHB119 cotton in comparison with all counterparts may nevertheless raise some question about this affirmative conclusion!!

Comment 2

Data is lacking on the levels and fate of the herbicide residues in plant tissues.
Although the effect of 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.
Rationale: the GM cotton plant is developed to be able to use the herbicides glufonisate ammonium and glyphosate. 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 seeds 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).

Comment 3

Not evaluated

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

The chance that GHB614 x T304-40 x GHB119 cotton will pose serious risks for toxicity is negligible. It is assumed that there is no biological pathway in which the newly-inserted genes would directly or indirectly interact with safety (Kok et al., 2014; Zdziarski et al., 2014). There is no plausible or testable hypothesis for an interaction of new proteins in GHB614 x T304-40 x GHB119 cotton (Steiner et al., 2013).

Comment 2

No questions

Comment 3
A 28 days repeated dose toxicity study with the protein Cry2Ae was performed. The results of the study showed no treatment-related changes.

No tests have been conducted with the other proteins as earlier test did not show any sign of toxicity. No further testing is needed.

The homology searches with known toxins are NOT up-to-date. The reports date from 2014.

Comment 4
The safety of the 2mEPSPS protein, PAT/bar protein, Cry1Ab, Cry2Ae protein were previously evaluated in the frame of the single events applications by the EFSA panel (EFSA scientific opinions published in the EFSA Journal). No further questions on this.
In the scope of the application of cotton GHB614 x T304-40 x GHB119 further up-to-date bioinformatics searches were performed for sequence homology between the AA sequences of the 4 proteins with known toxins. No further comments or questions.
We agree with the applicant that the 4 proteins have very specific activities, with different substrates and different pathway, different mode of action, different binding sites in insects. In addition there are no indications of additive, synergistic or antagonistic activities/effects observed in the comparative assessment (comparative analysis of composition).

Comment 5
None. This includes the analysis of possible interactions between newly expressed proteins (page 94 of Main dossier).

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not relevant

Comment 2
No comments, questions.

Comment 3
Not evaluated

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

Comment 1
No questions

Comment 2
Comparative analysis of composition
Conventional herbicide treatment
Test of difference
33/53 composition parameters were showing difference
28 were equivalent
2 showed equivalence more likely than not: **magnesium, sterculic acid**

1 was less likely equivalent: **fat**
2 were not equivalent: **dihydrosterculic acid, linolenic acid**
Test of equivalence
44 parameters, equivalence
4 parameters, more likely equivalence
2 parameters, less likely equivalence: **fat, palmitic acid**
2 parameters, non-equivalence: **dihydrosterculic acid, linolenic acid**

Intended herbicide treatment
Test of difference
36/53 composition parameters were showing difference
30 were equivalent
3 showed equivalence more likely than not: **ɣ tocopherol, sterculic acid, heptadecanoic acid**
2 were less likely equivalent: **fat, linolenic acid**
1 was not equivalent: **dihydrosterculic acid**
Test of equivalence
44 parameters, equivalence
4 parameters, more likely equivalence
3 parameters, less likely equivalence: **fat, palmitic acid, linolenic acid**
1 parameters, non-equivalence: **dihydrosterculic acid**

Fat, dihydrosterculic acid, palmitic acid, linolenic acid: The mean values were within the min/max range of the 6 non-GM commercial reference varieties and the reference range compiled from different literature sources.

In conclusion, the comparative analysis of composition did not identify relevant changes in the composition of cotton GHB614 x T304-40 x GHB119.
No further comments or questions.

Comment 3
Not evaluated

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

Comment 1
No questions

Comment 2
Not performed for the stacked event. No further testing is needed.

For each of the three single events GHB614, T304-40 and GHB119, a 90-day feeding study in rodents, is provided. No biologically relevant changes were observed.

Comment 3
No animal studies were performed with whole food/feed derived from cotton GHB614 x T304-40 x GHB119.
90-day rat whole food/feed studies were performed in 2010 with the single events GHB614 cotton, T304-40 cotton, GHB119 cotton for the single event applications (5 animals/cage, statistics on

individual animals, according to OECD guidelines for chemicals, common practice). The studies were not discussed in the EFSA scientific opinions of the single events applications as the studies were not performed according to the 2011 EFSA guidelines (2 animals/cage, cage as experimental unit). From a toxicological point of view I agree with the applicant that it is useless to perform another 90-day feeding study with 2 animals/cage. The applicant performed complementary statistics analysis for cage effects for the 3 single events.
The comparative analysis of composition did not identify relevant changes in the composition of cotton GHB614 x T304-40 x GHB119. Agreeing that it is not necessary to perform a 90-day rat whole food/feed study with the cotton GHB614 x T304-40 x GHB119.

Comment 4
Not evaluated

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
No questions

Comment 2
The stacked events lead to the combined expression of 2mEPSPS, PAT/*bar*, Cry1Ab and Cry2A proteins. All these proteins have been assessed individually before in the context of previous applications. No indications pointing towards an increased risk for allergenicity were then identified by EFSA. As these dossiers date back to 2008 - 2011 and new information on allergens has since then become available, the applicants updated the amino acid sequence homology comparison between the newly expressed proteins and known allergens using a 2014 database. The results of this updated analysis indicate that no biologically relevant sequence similarities are present between the 2mEPSPS, PAT/*bar*, Cry1Ab and Cry2A proteins and allergens listed in the 2014 databases. Finally, there are no indications that the sequences would be intrinsically unstable when stacked together by traditional breeding and/or engage in unintended interactions, hereby affecting the expression levels of the proteins. Accordingly, I agree with the applicant's conclusion that no concerns in relation to allergenicity of the (combined) newly expressed proteins were identified.

Comment 3
None

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

Comment 1
It is assumed that GHB614 x T304-40 x GHB119 cotton has no greater allergenic potential compared to conventional commercial cotton varieties, and that it does not pose a serious allergenic risk.

Comment 2

No questions

Comment 3
I have no further remarks.

Comment 4
Not evaluated

A.5.3. ADJUVANTICITY

Comment 1
No comments

Comment 2
I have no further remarks.

Comment 3
Not evaluated

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
No questions

Comment 2
Not evaluated

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

Comment 1
There is no reason to assume that the genetic modification may affect the nutritional value of the feed derived from GHB614 x T304-40 x GHB119 cotton based on the compositional equivalence.

Comment 2
No questions

Comment 3
Not evaluated

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
Table 1.6.1: no NHEL level is given for these 2mEPSPS, PAT/bar,Cry1Ab and Cry2Ae proteins? Not needed?

Comment 2

Not evaluated

C. RISK CHARACTERISATION

Comment 1
No questions

Comment 2
The conclusion on page 115: “In GHB614, T340-40 and GHB119 inserted sequences there are neither allergenic nor toxicological *in silico* findings associated with the presence of the putative ORF polypeptides or putative products of predicted genes.”, needs to be substantiated, having regard to my previous remark in section A.2.2.

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

Comment 1
No questions

Comment 2
None

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
A side effect of the use of genetically modified GHB614 x T304-40 x GHB119 cotton may be that it is not sustainable with regard to the pest management. Herbicide mixing (glyphosate and glufosinate in case of GHB614 x T304-40 x GHB119 cotton) exposes weeds to multiple mechanisms of action, which may delay resistance evolution. However, 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). Santos-Amaya et al. (2015) conducted laboratory selections of a *Spodoptera frugiperda* (Lepidoptera species) strain, which was already resistant to Cry1F maize with pyramided Bt maize expressing Cry1A.105 and Cry2Ab2 proteins. A *Spodoptera frugiperda* strain was resistant to the pyramided Bt maize after 10 generations of selection. This showed how rapidly resistance to pyramided Bt crops could occur once resistance/cross-resistance to one Bt gene is present. Carrière et al. (2015) mentioned that the concentration of each toxin of a two-toxin pyramid must be high enough to kill at least 95% of susceptible individuals for pyramids to be most effective. Furthermore, two-toxin pyramids are thus expected to be most effective when they kill at least 99.75% of susceptible insects, assuming that each toxin acts independently. In an analysis of nine pest–pyramid combinations, mortality on pyramids met this criterion in only half of the 18 observations. These authors stated that in many cases the survival of susceptible insects is greater than the threshold value of 0.25%, and cross-resistance occurs between the toxins in pyramided transgenic Bt crops.

Comment 2
No questions

Comment 3

None

E.2. General approach of the ERA

Comment 1
No questions

Comment 2
None

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 questions; risk estimate negligible

Comment 2
None

E.3.2. Plant to micro-organisms gene transfer

Comment 1
No question; risk estimate negligible

Comment 2
None

E.3.3. Interaction between the GM plant and target organisms

Comment 1
No questions

Comment 2
None

E.3.4. Interaction between the GM plant and non-target organisms (NTOs)

Comment 1
No questions: risk estimate negligible

Comment 2
None

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

Comment 1
GHB614 x T304-40 x GHB119 cotton is tolerant to glyphosate and glufosinate, which may result in an increased application of these specific herbicides. Health concerns with regard to the use of glyphosate have been reported (Mensah et al., 2015).

Comment 2
Not relevant

Comment 3
None

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
Not relevant

Comment 2
None

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
The new proteins in GHB614 x T304-40 x GHB119 cotton are unlikely to be detrimental for human and animal health. However, there is a side effect of the use of GHB614 x T304-40 x GHB119 cotton: glyphosate residues and its metabolite may be harmful for human and animal health.

Comment 2
No questions

Comment 3
None

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
Because of the controversy between the WHO (Guyton et al., 2015) and EFSA (EFSA, 2015) with regard to the safety of glyphosate, a new examination of glyphosate toxicity should be undertaken to adjust downward the acceptable daily intake for glyphosate, as proposed by Myers et al. (2016). Furthermore, the European Chemicals Agency is conducting an investigation into the wider human health effects of glyphosate: see http://echa.europa.eu/registry-of-submitted-harmonised-

classification-and-labelling-intentions/-/substance-rev/13201/term. In the meantime, the approval of GHB614 x T304-40 x GHB119 cotton may be postponed.

Comment 2
No comments

Comment 3
None

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1
No questions

Comment 2
None

E.4.2. Case-specific GM plant monitoring

Comment 1
No questions

Comment 2
None

E.4.3. General surveillance for unanticipated adverse effects

Comment 1
No questions

Comment 2
None

E.4.4. Reporting the results of monitoring

Comment 1
No questions

Comment 2
None

References

Carrière, Y., Crickmore, N., Tabashnik, B.E. 2015. Optimizing pyramided transgenic Bt crops for sustainable pest management. Nat. Biotechnol. 33, 161–168.

EFSA, 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA J. 13, 4302, 107 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. 72, 74-80.

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.

Kok, E.J., Pedersen, J., Onori, R., Sowa, S., Schauzu, M., De Schrijver, A., Teeri, T.H. 2014. Plants with stacked genetically modified events: to assess or not to assess? Trends Biotechnol. 32, 70-73.

Mensah, P.K., Palmer, C.G., Odume, O.N. 2015. Ecotoxicology of glyphosate and glyphosate-based herbicides - Toxicity to wildlife and humans. In: Toxicity and hazard of agrochemicals. Eds.: M.L. Larramendy and S. Soloneski, In Tech, Rijeka, Croatia, pp. 93-112.

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., vom Saal, F.S., Welshons, W.V., Benbrook, C.M. 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ. Health 15, Article 19 (13 pp).

Santos-Amaya, O.F., Rodrigues, J.V.C., Souza, T.C., Tavares, C.S., Campos, S.O., Guedes, R.N.C., Pereira, E.J.G. 2015. Resistance to dual-gene Bt maize in Spodoptera frugiperda: selection, inheritance, and cross-resistance to other transgenic events. Sci. Rep. 5, 18243 (10 pp).

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.

Zdziarski, I.M., Edwards, J.W., Carman, J.A., Haynes, J.I. 2014. GM crops and the rat digestive tract: A critical review. Environ. Int. 73, 423-433.