**Bioveiligheidsraad**
**Conseil de Biosécurité**

***24/05/2016***

**Secretariaat**
**Secrétariat**

O./ref.: WIV-ISP/41/BAC/2016_0356

**Title:** Advice of the Belgian Biosafety Advisory Council on the application EFSA-GMO-NL-2009-68 from Dow AgroSciences LLC under Regulation (EC) No. 1829/2003

## Context

The application EFSA-GMO-NL-2009-68 was submitted by Dow AgroSciences LLC on 19 March 2009 for the marketing of genetically modified (GM) cotton 281-24-236 x 3006-210-23 x MON88913 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1].

Cotton 281-24-236 x 3006-210-23 x MON88913 is a stacked event obtained by conventional crossing of the two-event stack cotton 281-24-236 x 3006-210-23 and MON88913. It expresses the Cry1Ac and Cry1F proteins conferring resistance to certain lepidopteran pests, and the PAT and CP4 EPSPS proteins conferring tolerance to glufosinate-ammonium and to glyphosate, respectively.

The application was officially acknowledged by EFSA on 3 March 2011. On 5 September 2013 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).

The Belgian Biosafety Advisory Council (BAC) did not participate in this consultation.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 9 March 2016 (EFSA Journal 2016;14(4):4430[2]), and published on 8 April 2016 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

In the frame of the preparation of this advice, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts chosen from the common list drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). The experts were invited to evaluate the dossier, taking also into account the EFSA opinion and the two advices already issued by the BAC on the events 281-24-236 x 3006-210-23[3] and MON88913[4].

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/4430
[3] Final advice of the Biosafety and Biotechnology Unit (SBB) on mandate of the Belgian Biosafety Advisory Council of 25 January 2011 on application EFSA/GMO-NL/2005/16 from Dow AgroSciences Europe (ref WIV-ISP/41/BAC_2011_0073)
[4] Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/UK/2007/41 from Monsanto under Regulation (EC) No. 1829/2003 (ref WIV-ISP/41/BAC/2014_0327)

Six experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of the comments.

The advice of the Biosafety Advisory Council given below is based on:

- The comments formulated by the experts
- The opinion of EFSA including the answer of the EFSA GMO Panel to the comments formulated by other Member States during the three-month consultation period
- The two advices already issued by the BAC on the events 281-24-236 x 3006-210-23 and MON88913. The conclusions were as follows:
  - For cotton 281-24-236 x 3006-210-23, the SBB concluded that the data presented are sufficient to indicate that no adverse effects are to be expected for human and animal health from the food, feed or industrial products. In addition, in case of accidental spillage, no unintended environmental effects are expected to occur.
  - For cotton MON88913, the BAC concluded that in the context of its intended uses, this GM cotton is unlikely to pose any risk to human and animal health. The BAC did not identify any risk that the import and processing of this GM cotton could pose to the European environment.

Cotton 281-24-236 x 3006-210-23 and cotton MON88913 are both authorised in the EU for food and feed uses with the exception of GMO cultivation[5].

## Scientific evaluation

### 1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of cotton 281-24-236 x 3006-210-23 x MON88913 seeds (i.e. during transport and/or processing) into the European environment[6] 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

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of cotton 281-24-236 x 3006-210-23 x MON88913, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

Cotton 281-24-236 x 3006-210-23 x MON88913 expresses four new proteins: Cry1Ac, Cry1F PAT and CP4 EPSPS. Based on previous assessments of these proteins and taking into account the information provided by the applicant, the Biosafety Advisory Council is of the

[5] EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=51 for cotton 281-24-236 x 3006-210-23 and http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=69 for cotton MON88913

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

opinion that in the context of its intended uses GM cotton 281-24-236 x 3006-210-23 x MON88913 does not raise safety concerns regarding toxicity.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that the newly expressed proteins in GM cotton 281-24-236 x 3006-210-23 x MON88913 may be allergenic under the intended conditions of use. It is also of the opinion that the simultaneous presence of these newly expressed proteins in the three-event stack cotton does not raise safety concerns regarding allergenicity.
Since the allergenicity of the whole GM cotton has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and shows the nutritional equivalence of GM cotton 281-24-236 x 3006-210-23 x MON88913 with its non-GM counterpart and conventional cotton varieties.

**4. Monitoring**

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

**Conclusion**

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the two advices already issued on the events 281-24-236 x 3006-210-23 and MON88913, and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM cotton 281-24-236 x 3006-210-23 x MON88913 is unlikely to pose any risk to human and animal health.

The Biosafety Advisory Council did not identify any risk that the import and processing of this GM cotton could pose to the European environment.

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


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

*Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2009-68 (ref. BAC_2016_0338)*

**Bioveiligheidsraad**
**Conseil de Biosécurité**

*18/05/2016*

**Secretariaat**
**Secrétariat**

O./ref.: WIV-ISP/41/BAC_2016_0338
Email. : bac@wiv-isp.be

**Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2009-68**

**Mandate for the Group of Experts:** Mandate of the Biosafety Advisory Council (BAC) of 20 October 2015.
**Coordinator:** Philippe Baret
**Experts:** Eddy Decuypere (KUL), 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, analysis of food/feed, food/feed processing, human and animal nutrition, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens.

**SBB:** Didier Breyer, Fanny Coppens, Katia Pauwels

## ♦ INTRODUCTION

Dossier **EFSA-GMO-NL-2009-68** concerns an application submitted by **Dow AgroSciences** for authorisation to place on the market genetically modified **cotton 281-24-236 x 3006-210-23 x MON88913** in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.
EFSA declared the application valid on 3 March 2011 and published its final opinion on this application on 8 April 2016 (EFSA Journal 2016;14(4):4430).

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 attention of the experts was drawn on the fact that this application concerns a GM plant containing a combination of three transformation events ("stacked transformation event"). The data for the single events have already been assessed by the Biosafety Council, leading to the following deliverables:
- Cotton 281-24-236 x 3006-210-23 (EFSA-GMO-NL-2005-16): SBB advice (on mandate of the Biosafety Council) issued on 25/01/2011. This GMO is authorised for commercialisation in the EU since 21/12/2011;
- Cotton MON88913 (EFSA-GMO-UK-2007-41): Council's advice published on 21/05/2014. This GMO is authorised for commercialisation in the EU since 24/04/2015.

Since comments from experts were requested after the publication of the EFSA's opinion, they were not sent to EFSA but rather used directly by the Biosafety Council as a scientific basis to draft its final advice on this application.

**List of comments/questions received from the experts**

## GENERAL COMMENTS

*Comment 1*

The 281-24-236 x 3006-210-23 x MON88913 cotton is a stacked product from parental lines expressing Cry1F, Cry1Ac, CP4-EPSPS and PAT-proteins and the application is for importing cotton and seeds for processing and use as food and feed, not for cultivation.
No further questions.

*Comment 2*

The 3-stacked event 281-24-236 x 3006- 210-23 x MON88913 cotton was evaluated as a whole. This means that possible repercussions of the genetically modified cotton were taken into account, not only because of the presence of new proteins, but also because it may have implications for human and animal health by the presence of residues of the herbicides themselves, or their metabolites, arising from an increased use of glyphosate and glufosinate in 281-24-236 x 3006- 210-23 x MON88913 cotton.
Single events dealing with Cry1Ac, Cry1F, PAT and CP4 EPSPS proteins have already been assessed and EFSA concluded that they are safe for human and animal health. It is assumed that there is no plausible or testable hypothesis for an interaction of the newly-inserted proteins. Consequently, the genetic modification of 281-24-236 x 3006- 210-23 x MON88913 cotton is no reason to prohibit its import and processing in the EU.
An indirect effect from 281-24-236 x 3006- 210-23 x MON88913 cotton cannot be excluded due to an increased use of glyphosate and glufosinate. Some health concerns about glyphosate have been reported. Although 281-24-236 x 3006- 210-23 x MON88913 cotton is not intended for cultivation in the EU, it may increase the use of these herbicides where cultivation is allowed. As a consequence, imported cotton, destined for food and feed use, may contain residues of these herbicides and their metabolites. Therefore, the import of 281-24-236 x 3006- 210-23 x MON88913 cotton should be banned until new epidemiological and toxicology studies clearly demonstrate the safety of glyphosate and its metabolites for human and animal health and the environment.
Comment SBB:
The assessment of the safety of herbicides is not in the remit of the Biosafety Council (legal basis = cooperation agreement on biosafety and GMO Directives).

## A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

*Comment*

No questions.

## 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.

*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 questions.

*Comment 2*
No comment. The dossier describes the combination of events that have been approved by EU. It is shown that the inserts from the separate events are combined in a stable manner. The genes from the inserts are expressed as in the separate events.

## A.3. COMPARATIVE ASSESSMENT

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

*Comment 1*
No questions.

*Comment 2*
The application concerns cotton, obtained by conventional breeding of events 281-24-236 x 3006-210-23 cotton and MON88913 cotton. This cotton has already been evaluated by EFSA.

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

*Comment 1*
No questions.

*Comment 2*

Why is only glyphosate used for the herbicide management, whereas 281-24-236 x 3006-210-23 x MON88913 cotton is also tolerant to glufosinate-ammonium?

Comment SBB:

The PAT gene was used as selectable marker in the development of the 281-24-236 and 3006-210-23 lines. However, according to the information provided by the applicant, tolerance to glufosinate ammonium in the GM plant is not high enough to be used under field conditions.

*Comment 3*

The comparator chosen for the evaluation of 281-24-236 x 3006-210-23 x MON88913 cotton consisted of non-genetically modified cotton with a comparable genetic background.

No further remarks

### A.3.3. COMPOSITIONAL ANALYSIS

*Comment 1*

As for mineral analysis of cottonseed, Sulfur and Molybdenum are out of literature range values; sulphur is higher and Mo lower.

S-comparisons between control and transgenic cotton reveal to be very similar (table 8, p.80), but no explanation is given why so much higher then literature values; although perhaps not strictly needed in the framework of this application it would be worthwhile to know why.

Moreover, why is neither S nor Mo included in table 8-1? Since just for these two elements the levels for control and the transgenic cotton fall outside values of literature range.

Why in this table (8.1), the values of controls for linolenic acid are so different of control 2005 and 281-24-236 x 3006-210-23 x MON88913 of 2005? (see columns 2, 4, 5, 7 versus 9 and 10, p.86).

I can agree with the composition summary that values for 281-24-236 x 3006-210-23 x MON88913 cotton composition samples from sprayed and unsprayed treatments were either statistically similar to control OR within literature ranges for cotton (not AND!).

Comment SBB:

According to the data provided by the applicant Sulfur results for the GM and control entries were very similar (<4% difference from the control entry). Although the data felt indeed outside of the literature range, it is very unlikely that this deviation from the reported values is caused by the transgenes. It should be noted that the same observation (values outside of the literature range for GMO and control) was made made for application EFSA-GMO-NL-2005-16 (parent event 281-24-236 x 3006-210-23).

Results for Molybdenum were near or below the limit of quantitation for the method, and the literature range provided for this mineral is available from only 1 literature source.

Results for linolenic (C18:3) for both the control and 281-24-236 x 3006-210-23 x MON88913 cotton samples were indeed slightly higher than the literature range and than data for the parent events presented in applications EFSA-GMO-UK-2007-41 and EFSA-GMO-NL-2005-16. However, since no statistical differences were observed between the GMO and the control in the current application, it is very unlikely that this deviation from the reported and previous values is caused by the transgenes.

*Comment 2*

For the across-site analysis, all values were within literature ranges with the exception of sterculic acid, which was slightly below the range for the unsprayed and sprayed 281-24-236 x 3006-210-23 x MON88913 cotton entries.

No significant differences between 281-24-236 x 3006-210-23 x MON88913 cotton and control entry results were observed in the cross-site analysis, except for sterculic and malvalic acid (lower levels of these anti-nutrients in transgenic).
Lower concentrations are not problematic.

*Comment 3*
Some compounds analysed in 281-24-236 x 3006- 210-23 x MON88913 cotton were different from non-GM cotton. However, mean values were within the range cited in the literature and the absolute differences between them were minor, so that differences are not of biological relevance.
The concentrations of sulphur in the control as well as in 281-24-236 x 3006- 210-23 x MON88913 cotton were higher than reported in the literature. Is it due to the experimental design or to the crop management? It is desirable to explain these high values.
Comment SBB:
According to the data provided by the applicant sulfur results for the GM and control entries were very similar (<4% difference from the control entry). Although the data felt indeed outside of the literature range, it is very unlikely that this deviation from the reported values is caused by the transgenes.

*Comment 4*
Compounds selected for analysis were proximates, minerals, vitamins, amino acids, fatty acids and anti-nutrients.
- Proximates: ash, fat, moisture, crude fibre, detergent fibre.
- Minerals: all relevant minerals are included.
- Vitamins: a detailed analysis of vitamin levels included vitamin A, B2, B5, B6, C, folate, niacin, and tocopherols.
- Fatty acids: saturated, mono-unsaturated and poly-unsaturated fatty acids are assessed.
- Amino acids: essential and non-essential amino acids have been studied.
- Anti-nutrients: the levels of sterculic, malvalic and dihydrosterculic acids have been determined. Gossypol was also determined including free and total gossypol.
- In the range of mycotoxins, aflatoxins have been studied.
As an overall conclusion the applicant states that the composition of cotton 281-24-236 x 3006- 210-23 x MON88913, sprayed and unsprayed, was always either statistically indistinguishable from the cotton background or within literature data for cotton.
The composition of this GM cotton has been shown to be equivalent to conventional cotton lines.
I agree with this conclusion.

### A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

*Comment 1*
No questions.

*Comment 2*
The applicant evaluated the agronomic traits of cotton 281-24-236 x 3006- 210-23 x MON88913 and concluded that this GM cotton is significantly comparable to other cotton.
No further remarks.

### A.3.5. EFFECTS OF PROCESSING

*Comment 1*
No questions.

*Comment 2*
Cotton 281-24-236 x 3006- 210-23 x MON88913 will be processed according to conventional methods. No novel processing technology is envisaged.
I agree with this conclusion.
Due to the equivalence in composition no particular effects of processing are to be expected.

## A.4. TOXICOLOGICAL ASSESSMENT

### A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

*Comment*
No questions.

### 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*
No questions.

*Comment 2*
The chance that the new proteins of 281-24-236 x 3006-210-23 x MON88913 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 281-24-236 x 3006-210-23 x MON88913 cotton (Steiner et al., 2013).

### A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

*Comment 1*
No questions.

*Comment 2*
The Cry1Ac, Cry1F and PAT proteins are readily degradable in simulated digestive juice.
Earlier studies demonstrated:
- The acute oral LD50 was greater than 375 mg/kg for Cry1F and 350 mg/kg for Cry1Ac.

- The acute LD50 of PAT protein could not be determined other than estimated to be higher than 5000 mg PAT per kg body weight.
For CP4 EPSPS, earlier studies demonstrated rapid degradation and lack of toxic effects.
No up-to-date searches are present.

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

*Comment*
No questions.

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

*Comment 1*
The safety of the newly expressed proteins has been shown previously for the single events of this stacked product.
Since there is compositional equivalence of the stacked event to the conventional cotton, no animal feeding studies are necessary.

*Comment 2*
90-day rat feeding study not performed.
No further testing is needed

### 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 allergenic potential of the Cry1F, Cry1Ac, CP4-EPSPS and PAT proteins has been assessed before according to EFSA recommendations. The results from these assessments confirmed that the Cry1F, Cry1Ac, EPSPS and PAT proteins do not pose any significant risk of being potential allergens.
The combination of the individual events in the three-event stack cotton, 281-24-236 x 3006-210-23 x MON88913, has been the subject of a recent EFSA scientific opinion (EFSA Journal 2016;14(4): 4430) which concluded that “no reasons of concerns regarding the simultaneous presence of these newly expressed proteins in the three-event stack cotton affecting allergenicity were identified”. No new experimental evidence has come forward questioning this conclusion.

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

*Comment 1*
No questions.

*Comment 2*
It is assumed that 281-24-236 x 3006- 210-23 x MON88913 cotton does not pose a serious allergenic risk, and that it is comparable with conventional cotton with regard to allergenicity.

*Comment 3*
In the same scientific opinion it was concluded that "the GMO Panel identified no indications of a potentially increased allergenicity of the three-event stack cotton 281-24-236 x 3006-210-23 x MON88913 food and feed with respect to non-GM cotton." I agree with this conclusion.

### A.5.3. ADJUVANTICITY

*Comment 1*
No questions.

*Comment 2*
No remarks.

## A.6. NUTRITIONAL ASSESSMENT

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

*Comment*
No questions.

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

*Comment 1*
No questions.

*Comment 2*
Based on the compositional equivalence and the fact that differences are not biologically relevant (see A.3.3), there is no reason to assume that the genetic modification has affected the nutritional value of the feed derived from 281-24-236 x 3006- 210-23 x MON88913 cotton. However, some caution is desirable with regard to sulphur. Maximum tolerable levels of sulphur in the feed (% of dry matter) amount to 0.4% for pigs and 0.3-0.5% for cattle and sheep (NRC, 2005). Sulphur in excess of 0.2% of dietary dry matter may have detrimental effects on performance of cattle (Zinn et al., 1995).

## B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

*Comment*
No questions.

## C. RISK CHARACTERISATION

*Comment*
No questions.

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

*Comment*
No questions.

## E. ENVIRONMENTAL RISK ASSESSMENT

### E.1. INTRODUCTION

*Comment*
No questions.

### E.2. GENERAL APPROACH OF THE ERA

*Comment*
No questions.

### 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*
No questions.

### E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

*Comment*
No questions.

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

*Comment*
No questions.

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

*Comment*
No questions, not applicable.

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

*Comment 1*
Not applicable.

*Comment 2*
281-24-236 x 3006- 210-23 x MON88913 cotton is not intended for cultivation in the EU. Nevertheless, the introduction of glyphosate-tolerant crops may result in the accumulation in soils of glyphosate and its metabolite (aminomethylphosphonic acid) in regions where its cultivation is allowed, so that the sustainability of genetically modified glyphosate-tolerant crops is questionable (Mamy et al., 2010; Mortensen et al., 2012).
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. Glyphosate use has risen almost 15-fold since genetically modified glyphosate-tolerant crops were introduced in 1996 (Benbrook, 2016).
281-24-236 x 3006- 210-23 x MON88913 cotton may have consequences in countries where its cultivation is allowed. 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 of herbicides and metabolites in plant tissues (Reddy et al., 2008; Bøhn et al., 2014) and surface water (VMM, 2015). Health concerns with regard to the use of glyphosate (Guyton et al., 2015; Seneff et al., 2015) and glufosinate (Laugeray et al., 2014) have been reported.

The application of these herbicides in weed management should meet the restrictions specific to herbicide-treated crops. Total glyphosate equivalents residue in seed amounted to 1.49 µg/g (Annexes; Phillips et al., 2008a), which is an indication of a appropriate management. Herbicide mixing 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 (Mortensen et al., 2012; Evans et al., 2015).

Comment SBB:

The assessment of the safety of herbicides is not in the remit of the Biosafety Council. Moreover, as mentioned by the expert, the scope of the current application does not include cultivation in the EU. Therefore the assessment of the possible impact of the application of glyphosate is not relevant in this context.

### E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

*Comment*

Not applicable.

### E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

*Comment 1*

No questions.

*Comment 2*

The new proteins in 281-24-236 x 3006- 210-23 x MON88913 cotton are unlikely to be detrimental for human and animal health. However, there is a side effect of the use of 281-24-236 x 3006- 210-23 x MON88913 cotton: glyphosate residues and its metabolite and glufosinate residues may be harmful for human and animal health, although total glyphosate equivalents residue in seed was rather low in the current dossier.

Comment SBB:

The issue of residues of the complementary herbicides and their metabolites is not in the remit of the Biosafety Council.

### E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

*Comment 1*

No questions.

*Comment 2*

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). In the meantime, the approval of 281-24-236 x 3006- 210-23 x MON88913 cotton for import and processing in the EU should be delayed.

Comment SBB:

The assessment of the safety of herbicides is not in the remit of the Biosafety Council.

### 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.

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