Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0141

Title: Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/NL/2011/97 from Bayer CropScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2011/97 was submitted by Bayer CropScience AG on 7 April 2011 within the framework of Regulation (EC) No. 1829/2003[1] for authorisation of insect-resistant and herbicide-tolerant genetically modified (GM) cotton T304-40 for import and processing for food and feed uses. Cotton T304-40 contains a single insert expressing the Cry1Ab and PAT proteins conferring resistance to certain lepidopterian pests and tolerance to glufosinate ammonium-based herbicides respectively.

The application was officially acknowledged by EFSA on 24 October 2011. On 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 GM organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Four experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 24 January 2012.

The opinion of the EFSA GMO Panel was adopted on 30 May 2013 (EFSA Journal 2013; 11(6):3251[2]), and published together with the responses from the Panel to comments submitted by the experts during the three-month consultation period.

On 21 June 2013 the EFSA opinion was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. The comments formulated by the experts together with the EFSA opinion including the answers of the EFSA GMO Panel, form the basis of the advice of the Biosafety Advisory Council given below.

[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/3251.htm

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment[3].

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 is of the opinion that the composition of the GM cotton T304-40 is compositionally equivalent to its conventional counterpart.

3.2. Assessment of toxicity

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

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that the newly expressed Cry1Ab and PAT proteins in GM cotton T304-40 may be allergenic.
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 the GM cotton 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.

[3] Since this application does not imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the EFSA opinion, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the questions of the EFSA GMO Panel and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM cotton T304-40 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.

p.o. Dr. Thierry Hermans, SBB
Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2011/97 and *comments submitted on the EFSAnet (ref. BAC_2012_0086)*

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24-01-2012

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/97 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 16 November 2011
Coordinator: Prof. Philippe Baret
Experts: Eddy Decuypere (KUL), Jean Jacquemin (CRA-Gembloux), Hadewijch Vanhooren (KUL), Johan Van Waes (ILVO)
Domains of expertise of experts involved: Molecular characterisation, human & animal nutrition, toxicology in vivo & in vitro, agronomy, ecology, herbicide tolerance, impact on bio-diversity, cotton
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2011/97** concerns an application of the company **Bayer CropScience** for the marketing authorisation of the genetically modified **T304-40 Cotton** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 24 October 2011.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If

information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

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

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

According to the dossier the scope of application does not include the authorization for the cultivation of cotton T304-40 seed products in the EU. It can however be worthwhile to give some remarks on the different topics, dealing with cultivation and survivability of seeds, in the case that the applicant should ask in the near future for an extension for the scope of cultivation, especially for cultivation in some southern European countries.
So as agronomical expert I will also give some comments in this questionnaire, related to cultivation and the environmental aspect.

Comment 2

The trypsin-resistent core protein of CryiAb from *B. thuringiensis* damages gut lining of lepidopteron larvae, leading to its destruction, and the larvae stop feeding. The combined effect of starvation and tissue damage cause death of larvae.
The bar coding sequence encodes a specific enzyme, PAT (phosphinotricin acetyl-transferase) that acetylates the herbicide glufosinate ammonium and thereby detoxifies this herbicide. The working mechanism of the insect-resistant and glufosinate-tolerant T304-40 cotton is well explained.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

Comment 1

Under “3. Survivability – a) Ability to form structures for survival or dormancy” it is mentioned that “Cultivated cotton does not produce seeds which can persist in the environment for long periods of time, furthermore cotton seed lacks the ability to develop dormancy." My question is : Are there data available to prove this?
Additional comment SBB
The comment above or similar comments on the survivability of cotton seed were submitted to EFSA for applications 41, 42 and 51. This issue was also addressed in the BAC advice for application 51.
It should be noted that for the current dossier, the applicant provided some information about dormancy, survivability and over wintering of cotton T-304-40 in Southern Europe (see page 56 of

technical dossier). A General Surveillance will be also undertaken during the authorisation period for import and processing. This monitoring system will involve the authorisation holder and operators handling and using viable T304-40 cotton seed.

Comment sent to EFSA (provided under D.11.4)

According to the Biosafety Advisory Council the main potential risk concerning the environment relates to the potential establishment of feral populations in case of unintentional release into the environment of GM cotton seeds during transportation and processing. Establishment of feral populations in case of incidental spillage is very unlikely to occur in Belgium and in Northwest Europe in general because the climate in these regions is not suited for cotton growth. On the other hand, the possibility of seed spillage and seed germination with a further establishment of feral populations exists in Southern Europe.

The Biosafety Advisory Council agrees that the agronomic assessment does not give any indication to assume that cotton T304-40 has an increased survivability compared to conventional cotton lines. Moreover, cotton T304-40 will be imported as mostly non-viable seed which makes the likelihood that some imported seed could escape and germinate very low.

Nevertheless, the Biosafety Advisory Council supports the view that appropriate management systems should be in place to minimize accidental loss and spillage of transgenic cotton during transportation, storage and handling in the environment and processing into derived products. In addition, the Council is of the opinion that the general surveillance should include specific measures to actively monitor the occurrence of feral cotton plants in areas where seed spillage and plant establishment are likely to occur where climatically appropriate (such as harbours, transit road-sides and vicinity of processing plants). We are keen to know the results of these surveillance plans for the previous cotton dossiers.

Comment 2

P. 21: What is the positive Halphen test?

Comment 3

The botanical and agronomic characteristics are presented with some considerations on the worldwide cotton production and also on the gossypol problem.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments or questions; well explained.

Comment 2

The 2 new genes introduced are described. The *cry1Ab* and *bar* genes are already in use for several years. Some regulatory agencies have already authorized these traits for human and animal consumption.

Plasmid map is well described and promoters and genes are positioned. Two tables summarize the genetic elements in the vector and those introduced into the plant.

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Several tables summarize the experimental approach to the characterization of the T304-40 cotton, at the molecular level, the field trials, the composition and toxicology.
The new traits: insect resistance and herbicide are explained. Meanwhile, the exact nature of the truncated *cry1Ab* gene is not indicated in this part of the dossier.
Comment sent to EFSA
The inserted DNA sequence in T304-40 cotton has been fully determined and is described in Moens and De Pestel, 2008. Some information about the Cry1Ab protein is given on page 10 of the dossier. Further data must be retrieved from other sources, such as EPA (US) and FSANZ (Food Standards Australia New Zealand). We assume that the Cry1Ab protein encoded by the gene inserted into cotton T304-40 contains 617 amino acids and has a molecular weight of approximately 69 kDa, and that the deduced amino acid sequence is identical to the native Bt protein now known as Cry1Ab5 (Höfte et al., 1986), except that it is truncated at the C-terminal end (the Bt produced protein has a molecular weight of 130 kDa), and an alanine has been inserted at position 2 of the N-terminal end. Can you confirm that these assumptions are correct? What is the rationale for using a truncated version of the protein?

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The number, the integrity and the stability of the insertions in T304-40 cotton are analysed in details. The restriction map of the inserted sequence is shown and a schematic drawing of the T304-40 insert allow the presentation of the potential fragments obtained with the different restriction enzymes used in this part of the dossier. Seven Southern blots hybridized to different parts of the plasmid described

the composition of the inserts into the cotton plant. Some fragments are missing in the screening but all the potential fragments are summarized in table 12 and 13. Missing fragments in the blots can be explained by the small size of the insert to detect.
The inserted genetic material was amplified in 6 fragments by PCR reactions and the different products were sequenced and aligned. DNA sequence of the insert is identical to the original transforming plasmid except a change of one amino acid in the 3'me1 terminator.
Bioinformatic analysis were performed on this sequence for ORF, promoter and Start sites. Bioinformatic analysis were conducted in the pre insertion locus and it was concluded that it is unlikely that new ORF would be expressed or that known genes can be interrupted.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The expression of the new genes *cry1Ab* and *bar* were monitored in plants at different growth stages by Elisa method. Younger leaves contain higher level of cry1Ab and PAT proteins that older leaves .
The expression was also estimated in seeds harvest from field trials. The level observed in cotton sprayed or not with glufosinate ammonium is not different and therefore herbicide treatment does not have effect on the expression of PAT and cry1Ab proteins.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No change was observed in fertility, in fecundity and in dormancy. No seed remains viable after 7 months in the soil during winter.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Genetic stability was extensively tested by multiple conventional crosses over some years. Genetic stability was demonstrated in 4 Southern blots on different generation issued from crosses with cotton T304-40. A digestion with EcoRV restriction enzyme give the 3 expected fragments with the genomic DNA extracted from the different plants. The new phenotype was also investigated by spraying plants with the herbicide. The Chi square test was significant for Mendelian inheritance.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No transfer to bacteria is expected as the promoters are not functional in bacteria.

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

- P. 67: Why only 8 locations given whereas 12 sites were used? I understand that at the time when the trials were planned and performed in Spain, EFSA guidance on how many sites were required for compositional analysis was not yet available or not clear, and hence 12 sites were realized.
But on what basis these 8 sites were selected out of the 12?
- What means “good internal quality control” as a basis of selection of sites for further analysis?
- Choice of comparator, Coker 315, is ok.

Additional comment SBB
We have checked the compliance of the compositional analysis provided by the applicant to the OECD recommendations (2009) and everything seems OK.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

It is mentioned (p. 72) that a complete randomized block with 3 replications and 3 treatments is used, and the 3 treatments are mentioned in A), B) and C). However 6 lines further it is mentioned that the samples represented regimens sprayed with glufosinate ammonium herbicide and unsprayed transgenic and non-transgenic counterparts. In Fig. 21 A and B are sprayed with conventional herbicides, which is not the same as “unsprayed”.
Why only these 3 treatments considering only the glufosinate and conventional herbicide?
Why not conventional treatment and T304-40 with glufosinate, with or without insecticide, or A) B) C) with or without insecticide? This would be then 6 treatments.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

All sites showed significant differences in composition of cottonseed for myristic acid, palmitoleic acid and stearic acid between T304-40 cottonseed and its non GM counterpart (USA trial). However, amounts are within the range given in literature, and it concerns relatively small differences of minor fatty acids, so it has no consequences for nutritional equivalence, but any idea why these differences? No hypothesis or possible explanation is given.
Since the same fatty acids are also different (p. 86 and table 30) in EU field trials (for most of the sites at least), it would be worthwhile to speculate on this from a scientific point of view.
P. 36, table 30, 31: Why these consistent higher Ca levels in GM cottonseed?

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No questions.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

- To complete the assessment of toxicity of both proteins (Cry1Ab and PAT) in mice, doses of respectively 2000mg/kg BW and 10 mg/kg BW via parental route or for oral gavage were used: why? These doses are not related to the protein contents in GM-cotton seed of both proteins: for Cry1Ab it was 1.77-3.38 microg/g FW and for PAT, 97.8-222 microg/g FW.
This is a factor of ±100 difference, but in the reverse sense of the concentrations used for acute toxicity or safety assessment. Can this be clarified?
- What is the meaning of aprotinin as negative control and melitin as positive control?

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Newly expressed proteins: Cry1 Ab protein, PAT protein (*bar* gene).
The assessment of both proteins is adequate and acceptable. No further comments/questions.

Comment 1

No questions.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comments/questions.

Comment 2

No questions.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No particular natural constituents of T304-40 cotton are considered to be of significant concern to require additional information or further risk assessment. No further comments/questions.

Comment 2

No questions.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Repeated-dose 90-day oral toxicity in the rat (Totis, 2010), with 10% toasted cottonseed meal
The assessment is adequate and acceptable.
Nevertheless, from the report it is not clear if the T304-40 cotton was grown with glufosinate ammonium herbicide weed control (and which spraying regime was used).
42-day poultry feeding study (Stafford, 2010), with 10% toasted cottonseed meal
The assessment is adequate and acceptable.
The spraying regime of the glufosinate ammonium herbicide weed control was clearly described.

In conclusion: no potential health and food/feed safety concerns have been identified.

Comment 2

The overall conclusion that the data demonstrated that the safety data obtained using the bacterial-produced Cry1Ab and PAT proteins could be used for support of the safety of the plant-produced Cry1Ab and PAT proteins, is warranted.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

No questions.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

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

Comments/Questions of the expert(s)

Comment 1

Poultry were selected in order to evaluate the effects of the food component over an entire life span, but it would be better to use the term “economic life span” since it is considering the 6 week-growing period of broilers until slaughter weight, but this is by no means the entire biological life span which is rather 1-2 years or more.

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that “the likelihood that some escaped seed would germinate is very low because most of the imported seed is non-viable." My question is: Is the germination power of the imported seed analysed?

Additional comment sent to EFSA

What is the process used to make the seed "non-viable"? What is the real proportion of viable and non-viable imported seeds? Can one restrict import to non-viable seeds?

Comment 2

Not applicable.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not applicable.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

Not applicable.

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

Comments/Questions of the expert(s)

Comment 1

In this paragraph it is mentioned that the scope of the present application does not include cultivation of cotton plants in the EU and is limited to import and processing. Nevertheless I give here some remarks in the case that the applicant should ask in the near future for an extension for the scope of cultivation. In the framework of the EU regulation 2002/53 a new variety has to be submitted to DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use) tests before the variety can be commercialised. The new variety has to be compared with the best existing standard varieties.

So my question here is : Can the GM cotton be incorporated in normal VCU trials, for example treated with specific herbicides for cotton and will the agronomical value be the same as tested in trials, where the herbicide glufosinate ammonium, for which the variety is tolerant, is used?
Additional comment SBB and coordinator
Same comment was made for application 51 and was NOT sent to EFSA.

Comment 2

Not applicable.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Not applicable.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No questions.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Based on the scope of application (no cultivation) I can agree with the remark that the overall environmental risk posed by this genetically modified plant is negligible in the context of the intended uses of cotton T304- 40.

Comment 2

No questions.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No questions.

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

Comments/Questions of the expert(s)

Comment 1

No questions.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No questions.

References

None