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Conseil de Biosécurité

21-05-2014

Secretariaat
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O./ref.: WIV-ISP/41/BAC/2014_0327

Title: Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/UK/2007/41 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2007/41 was submitted by Monsanto on 11 April 2007 within the framework of Regulation (EC) No. 1829/2003[1] for authorisation of herbicide-tolerant genetically modified (GM) cotton MON 88913 for import and processing, and for food and feed uses. Cotton MON 88913 contains a single insert expressing the CP4 EPSPS protein conferring tolerance to glyphosate-based herbicides.

The application was officially acknowledged by EFSA on 19 October 2007. 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). Seven experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annexes I and II for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 18 January 2008.

The opinion of the EFSA GMO Panel was adopted on 3 July 2013 (EFSA Journal 2013; 11(7):3311[2]), and published together with the responses from the Panel to comments submitted by the experts during the three-month consultation period. This opinion was complemented on 26 February 2014 by an EFSA statement (EFSA Journal 2014; 12(3):3591[3]) taking into consideration updated bioinformatic analyses provided by the applicant.

On 31 July 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, and the complementary EFSA statement, 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/3311.htm
[3] See http://www.efsa.europa.eu/en/efsajournal/pub/3591.htm

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment[4].

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 GM cotton MON 88913 is compositionally equivalent to its conventional counterpart.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the CP4 EPSPS protein in the context of several previous applications, and no concerns were identified. Taking into account the information provided by the applicant, the Council is of the opinion that this conclusion remains valid and that the assessment of the toxicity of GM cotton MON 88913 does not raise safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the CP4 EPSPS protein in the context of several previous applications, and no concerns were identified. Taking into account the information provided by the applicant, the Council agrees with the EFSA GMO Panel that there are no indications that the newly expressed CP4 EPSPS protein in GM cotton MON 88913 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.

[4] 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 and the complementing statement, 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 MON 88913 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

23-5-2014

Annexes I and II: Compilation of comments of experts in charge of evaluating application EFSA/GMO/UK/2007/41 (ref. BAC_2008_PT_637) and comments submitted on the EFSAnet (ref. BAC_2008_PT_638)

Bioveiligheidsraad
Conseil de Biosécurité

January 2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2008/PT_637
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2007/41

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 9 December 2007
Coordinator: Prof. Philippe Baret
Experts: Pascal Cadot (Consultant), Leo Fiems (ILVO), Peter Smet (Consultant), Wim Stevens (UIA), Frank Van Breusegem (VIB), Jan Van Doorselaere (KH Zuid-West Vlaanderen), Johan Van Waes (ILVO)
Domains of expertise of experts involved:
Genetics, genetic engineering, molecular characterisation, transgene expression, bioinformatics, toxicology, immunology, alimentary allergology, animal nutrition, agronomy, breeding, improvement of plants, herbicide tolerance, ecology, nature conservation, bio-diversity, invasive species, ecotoxiclogy, entomology, soil-plant interactions, herbicide tolerance, transgene expression, biosafety, risk analysis.
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/41** concerns an application of the company **Monsanto** for the marketing of the genetically modified **cotton MON88913** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 October 2007.
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.

List of comments received from the experts

A. GENERAL INFORMATION

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 MON 88913 cotton seed products in the EU. It can however be worth 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 fact that MON 88913 was developed using the same CP4 EPSPS coding sequence and chloroplast targeting sequence as MON 1445, whose safety and substantial equivalence to conventional cotton (except for the introduced glyphosate-tolerance trait) have been established previously, may be favorable with regard to the evaluation of the application of MON 88913.

Comment 3

No comments / questions

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

Comments/Questions of the expert(s)

Comment 1

Under "3. Survivability – specific factors affecting survivability" it is mentioned it is highly unlikely that cottonseed would overwinter and germinate the following spring. My question is : are there data available of overwintering of seed of cotton?

Comment 2

No comments / questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

P 21-29 of the technical dossier: No comments.

Comment 2

No comments / questions

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

No comments / questions

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

Comments/Questions of the expert(s)

Comment 1

P 30-50 of the technical dossier:

Solid description of the work.
How did authors obtain flanking DNA sequences?
What is the chromosomal position of the insert?

The conclusion on p 38 concerning the BlastX search is correct (… the insertion did not disrupt any known ORF…). A suggestion for EFSA could be to include (in the future) some (routine) techniques (such as microarrays and proteomics) to analyze the GMO in question for changes in gene expression (due to the insertion of a foreign gene).

Comment 2

No comments / questions

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

p 51-57 of the technical dossier:

Provide a western blot demonstrating that monoclonal Antibody 39B6 recognizes 1 protein of 43 kDa

What is the definition of LOD (3x STD of the blanc?) and LOQ (10 x STD of the blanc)? Why is LOD bigger than LOQ?

No further comments.

Comment 2

No comments / questions

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

Remarks concerning the survivability of seeds of cotton. In the dossier it is mentioned that seed of cotton is known to be a weak competitor in the wild, which can not survive outside cultivation without human intervention. Furthermore the applicant mentioned that field observations have demonstrated that MON 88913 has not been altered in its survival, multiplication and dissemination when compared to conventional cotton varieties. My question is : are there real data to prove that in no cases survival of seed, after optimal conditions in the field during winter, were observed.

Comment 2

No comments / questions

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

Comments/Questions of the expert(s)

Comment 1

P 77-81: On p78 it is said that the MON 88913 –R3 and R4 lines are used to create commercial varieties; it would be informative if some data are provided on the final commercial lines (data in terms of T-DNA intactness and expression of the CP4 EPSPS)

No further comments.

Comment 2

No comments / questions

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

Comments/Questions of the expert(s)

Comment 1

No comments / questions

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that MON 88913 was compared to other commercial conventional cotton varieties. What does it mean? The MON 88913 is tolerant to glyphosate. So I think it is not possible to compare with commercial conventional varieties, unless they are also tolerant to glyphosate (= are also genetically modified). My question is : Is MON 88913 compared to other genetically modified varieties or only to conventional varieties and in the last case was the herbicide tolerance taken into account in this comparison?

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Is MON 88913 tested in 2002 and 2004 in comparison with other genetically modified varieties?

Comment 2

The CP4 EPSPS protein was produced by recombinant E. coli, because it may be practically impossible to obtain a sufficient amount of plant derived protein. The observed similarity in protein mobility and immunoreactivity for the plant- and *E. coli*-produced CP4 EPSPS proteins provides

evidence that the plant-produced CP4 EPSPS protein is equivalent to the *E. coli*-produced reference standard. Nevertheless, it has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and Schubert, 2004).

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

It is mentioned that field trials with MON 88913 were performed and that MON 88913 is equivalent to the conventional cotton, except for the introduced glyphosate-tolerance trait. Are the data of the 2 years of testing nearly the same?
From this information we can conclude that there is no significant difference in the agronomical value between the MON 88913 and the conventional type. Is this conclusion correct?

D.7.5 Product specification

Comments/Questions of the expert(s)

D.7.6 Effect of processing

Comments/Questions of the expert(s)

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

The CP4 EPSPS protein represents only a very small proportion in MON 88913 cottonseed as its concentration corresponds to only about 0.12 % of the total protein in cottonseed.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

a) Degradation of the CP4 EPSPS protein in simulated gastric fluid and simulated intestinal fluid (Harrison *et al.*, 1996).

The data demonstrated a half life of CP4 EPSPS of less than 15 s in the gastric system and less than 10 min in the intestinal system, based on Western blot analysis. Enzymatic activity of CP4 EPSPS was not detected in the gastric system after 2 min, and less than 9% was detected after 285 min in the intestinal system.

Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al.*, 2003). So, is this latter protein the one being used in the study of Harrison *et al.* (1996)?

b) Degradation of the CP4 EPSPS protein in simulated gastric fluid (Leach *et al.*, 2002).

The results of this study demonstrate that the *E. coli*-produced CP4 EPSPS protein was rapidly digested after incubation in SGF. The SDS-PAGE colloidal blue gel staining method demonstrated that at least 98% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds. No degenerative bands due to digestion were observed. Western blot analysis confirmed that more than 95% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds. Likewise, it was demonstrated that the EPSPS activity was reduced by >90% within 15 seconds of incubation of the CP4 EPSPS protein in SGF.

Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al.*, 2003). So, is this latter protein the one being used in the study of Leach *et al.* (2002)?

c) Equivalency assessment of the CP4 EPSPS protein derived from a microbial expression system with the CP4 EPSPS protein derived from MON 88913 (Karunanandaa *et al*., 2003)

The levels of CP4 EPSPS protein in MON 88913 are very low. To obtain sufficient quantities of this protein for evaluation of food and feed safety, it was necessary to produce it by *E. coli* fermentation.
A panel of analytical tests was used to identify, characterize and compare the plant- and *E. coli*-produced CP4 EPSPS proteins: (1) Western blot analysis and densitometry, (2) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry, (3) matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS), (4) N-terminal sequence analysis, (5) glycosylation analysis and (6) CP4 EPSPS enzymatic activity assay.
Collectively, these data establish the identity of the plant-produced CP4 EPSPS protein and its equivalence to the *E. coli*-produced CP4 EPSPS.

d) CP4 EPSPS: Acute Oral Toxicity Study in Mice (Harrison *et al*., 1996)

The title "The Expressed Protein in Glyphosate-Tolerant Soybean, 5-Enolypyruvylshikimate-3-Phosphate Synthase from Agrobacterium sp. Strain CP4, Is Rapidly Digested In Vitro and Is Not Toxic to Acutely Gavaged Mice" indicates that this study was performed for another (similar) GM product.
Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al*., 2003). So, is this latter protein the one being used in the study of Harrison *et al.* (1996)?

Comment 2

The safe use of MON 1445 and of other glyphosate-tolerant crops, expressing similar CP4 EPSPS proteins (such as NK603 maize), suggest that MON 88913 is as safe and nutritious as conventional cotton. Because of the history of safe use and consumption of CP4 EPSPS protein and the low concentration of the introduced proteins in tissues that are consumed, MON 88913 may not have adverse effects on animal or human health.

Comment 3

P 119 – 125:
Although of little relevance it is not really clear why three different N-terminal sequences are obtained; did it concern three different batches of purified protein (maybe from different seed lots?)
No further comments.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day poultry feeding study

Not included.

b) 13-week feeding study in rats with cottonseed from MON 88913 (Kirkpatrick, 2005).

The study design included three groups of Sprague-Dawley rats consisting of 20 rats/sex/group. One group was administered a diet containing approximately 2% (w/w) MON 88913, supplemented with approximately 3% (w/w) of the control cottonseed, MON 88913(-). Another group was administered a diet containing approximately 5% (w/w) MON 88913. A control group received diets containing approximately 5% (w/w) MON 88913(-) on a comparable regimen. These diets were administered concurrently *ad libitum* for a minimum of 90 days.
All animals survived to the scheduled necropsy with the exception of one control group male. There were no test substance-related clinical observations. Body weights, food consumption, and clinical pathology parameters were unaffected by test substance administration. There were no test substance-related effects on organ weights or findings at the macroscopic or microscopic examinations.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

The genetic modification introduced in this cotton (Gossypium) plant is the introduction of the material to induce the plant to synthesise the CP4 EPSPS protein which is associated with glyphosate tolerrance MON 88913. This insert had already earlier been used as MON 1445. The increased level of tolerance to glyphosate is reached by the use of improved promoter sequences.
The allergenicity has been tested in different ways:

- comparison with amino acid sequences of known allergenic proteins
- study of stability in simulated gastric fluid
- evaluation the allergenicity of the CP4 EPSPS protein and the organism of origin (Agrobacterium sp): there was only 30.5 % identity in a strech of 82 AA with Dermatophagoides farinae Der f 2.

From the data presented it is concluded that there is no threat for allergenicity.
When searching the medical literature I found a paper by Hoff et al. (2007) reporting that there was no binding of patients allergic to soy, Dermatophagoides and controls to the CP4 EPSPS protein introduced in soy.
With an ELISA technique, developed to demonstrate IgE binding to a.o. CP4 EPSPS, no differences between “allergic” patients and controls were found (Takagi et al. 2006/182). Sten et al. (2004/21) were not able to detect any significant difference in the allergenic potency between GM and wild type soybeans, using in vitro methods (RAST inhibition and histamine release) and skin prick tests.
Although these studies were not performed with cotton, they do not point to an allergenicity of the CP4 EPSPS protein. Follow up of patients allergic to Dermatophagoides pteronyssinus and farinae seu culinae is mandatory.

Comment 2

Simulated gastric fluid (SGF) was used to test the digestion of CP4 EPSPS proteins. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain.

Comment3

Assessment of allergenicity of the introduced trait
CP4 EPSPS has been considered as allergy safe by EFSA scientific panel. To the knowledge of the reviewer, there is no new data that could contest this decision.

Assessment of allergenicity of the whole GM plant
The applicant did not evaluate the potential allergenicity of MON 88913 cottonseeds, compared to their traditional counterpart. The reviewer agrees, however, that cottonseed allergy is not a major issue, which means that a limited number of allergic sera can be available. Furthermore, no major allergen of cottonseed has been defined. However, because the introduction of a new trait might influence the expression levels of other proteins of the host plant, it might be useful to evaluate the content of 2S storage protein and of vicillin, two known common seed allergens, in the MON 88913 cottonseed.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

It is noted that the introduced trait is of agronomic interest and is not intended to change any nutritional aspects of this cotton. Can this be proved by data?

Comment 2

A feeding study in channel catfish was conducted to evaluate the nutritional value of the MON 88913 cottonseed. Energy metabolism in fish is similar to that in mammals and birds with two notable exceptions. These exceptions are: (a) fish do not expend energy to maintain a body temperature different from that of their environment; and (b) the excretion of waste nitrogen requires less energy in fish than it does in homeothermic land animals (Anonymous, 1980). Fish may differ from mammals in its myofibre type: fish has more ßW fibres which may result in lower maintenance requirements.
On the other hand, there is no reason to assume that the nutritional value of the MON 88913 cottonseed may be different from earlier GMO cottonseed (MON 1445).

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

In this chapter it is mentioned that the advantage is of purely agronomic interest and presents negligible risk to the non- agricultural environments because the poor survival characteristics under most European conditions. How must we interpret "negligible" (are there data available) and "poor survival characteristics" (this does not exclude that there is a possibility of survival if the conditions were favourable for example in Southern European countries) ?

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

The safety to humans has been established based on the lack of acute oral toxicity in mice.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

The safety to animals has been established based on the lack of acute oral toxicity in mice and the feeding study with catfish.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

Comment 1

In this paragraph it is mentioned again that the scope of application does not include cultivation of cotton plants in the EU. 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 have 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 glyphosate, for which the variety is tolerant, is used?

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The proposed environmental monitoring plan is OK .

Comment 2

Experimental results revealed no effects of the genetic modification that would result in increased pest potential or ecological risk of MON 88913 **(**Horaka et al., 2007).

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 and that no specific strategies for risk management and no case-specific post monitoring actions are considered required.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

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18/01/ 2008

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Application EFSA/GMO/UK/2007/41
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 9 December 2007
Coordinator: Prof. Philippe Baret
Experts: Pascal Cadot (Consultant), Leo Fiems (ILVO), Peter Smet (Consultant), Wim Stevens (UIA), Frank Van Breusegem (VIB), Jan Van Doorselaere (KH Zuid-West Vlaanderen), Johan Van Waes (ILVO)
Domains of expertise of experts involved:
Genetics, genetic engineering, molecular characterisation, transgene expression, bioinformatics, toxicology, immunology, alimentary allergology, animal nutrition, agronomy, breeding, improvement of plants, herbicide tolerance, ecology, nature conservation, bio-diversity, invasive species, ecotoxiclogy, entomology, soil-plant interactions, herbicide tolerance, transgene expression, biosafety, risk analysis.
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/41** concerns an application of the company **Monsanto** for the marketing of the genetically modified **cotton MON88913** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 October 2007.
The scope of the application is:
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☐ 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)

Comments posted on the EFSAnet

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). Below you will find the comments as they were forwarded to the EFSAnet, and in a separate document the compilation of all the comments that were given by the experts (including the references). Only those comments that raised a question or a concern were forwarded to the EFSAnet. The fact that comments that did not raise a question or a concern were not forwarded to the EFSAnet does not diminish the value of these comments. They are absolutely necessary for the complete analysis of the dossier, and will be used in formulating the final advice by the Biosafety Advisory Council.

A. GENERAL INFORMATION

No comments.

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

Comments/Questions of the expert(s)

Under “3. Survivability - specific factors affecting survivability”, it is mentioned it is highly unlikely that cottonseed would overwinter and germinate the following spring.
However, Belgian experts already concluded that, in the context of the foreseen use of such GM cotton, accidental spillage of cotton seeds will be unavoidable (see i.e. advice of the Belgian Biosafety Advisory Council on dossier EFSA/GMO/NL/2005/13 - LLCotton25). If it occurs in southern Europe, it is likely that cotton would establish a feral population.
Our questions are :
- Could the application provide relevant data over overwintering of cotton seeds demonstrating that it is highly unlikely that cotton seeds would overwinter and germinate the following spring in case of accidental spillage in the potential receiving environment in Europe?
- In case of establishment of feral population, what would be the impact on the receiving environment in terms of selective advantages or disadvantages?

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

No comments.

D. INFORMATION RELATING TO THE GM PLANT

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

No comments.

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

Comments/Questions of the expert(s)

A suggestion for EFSA could be to include (in the future) some (routine) techniques (such as microarrays and proteomics) to analyze the GMO in question for changes in gene expression (due to the insertion of a foreign gene).

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

No comments.

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

Comments/Questions of the expert(s)

Remarks concerning the survivability of seeds of cotton. In the dossier it is mentioned that seed of cotton is known to be a weak competitor in the wild, which can not survive outside cultivation without human intervention. Furthermore the applicant mentioned that field observations have demonstrated that MON88913 has not been altered in its survival, multiplication and dissemination when compared to conventional cotton varieties. My question is : Are there real data to prove that in no cases survival of seed, after optimal conditions in the field during winter, were observed.

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

Comments/Questions of the expert(s)

On p78 it is said that the MON88913 –R3 and R4 lines are used to create commercial varieties; It would be informative if some data are provided on the final commercial lines (data in terms of T-DNA intactness and expression of the CP4 EPSPS) –

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

No comments.

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)

In this chapter it is mentioned that MON88913 was compared to other commercial conventional cotton varieties. The choice of these varieties should be better documented and motivated.

D.7.2 Production of material for comparative assessment

No comments.

D.7.3 Selection of material and compounds for analysis

No comments.

D.7.4 Agronomic traits

No comments.

D.7.5 Product specification

No comments.

D.7.6 Effect of processing

No comments.

D.7.7 Anticipated intake/extent of use

No comments.

D.7.8 Toxicology

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

On page 150, the applicant states that "In addition to the long history of safe use associated with CP4 EPSPS ...". This type of sentence is completely irrelevant in a scientific dossier and denotes a lack of scientific quality of the dossier on this point. Indeed, to our knowledge, no epidemiological study was achieved to support such a conclusion on the effects of CP4 EPSPS. Asbestos and tobacco also had a "long history of safe use" before the epidemiological studies.
On the other hand, we are aware that a large amount of data is available supporting the safety assessment of CP4 EPSPS. The applicant is invited to complete the dossier by providing clear references to these data and by indicating their relevance in the context of the safety assessment of the current application.

Comment 2

a) Degradation of the CP4 EPSPS protein in simulated gastric fluid and simulated intestinal fluid (Harrison *et al.*, 1996).
Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al*., 2003). So, is this latter protein the one being used in the study of Harrison *et al.* (1996)?

b) Degradation of the CP4 EPSPS protein in simulated gastric fluid (Leach *et al.*, 2002).
Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al*., 2003). So, is this latter protein the one being used in the study of Leach *et al.* (2002)?

d) CP4 EPSPS: Acute Oral Toxicity Study in Mice (Harrison *et al*., 1996)
Are the proteins in the above mentioned study and the current application the same? It was demonstrated that the CP4 EPSPS protein isolated from MON 88913 is equivalent with the *E. coli*-produced CP4 EPSPS (Karunanandaa *et al*., 2003). So, is this latter protein the one being used in the study of Harrison *et al.* (1996)?

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

No comments.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

No comments.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

A 42-day poultry feeding study is not provided in the dossier. We assume that this is based on the fact that the comparative analysis between the GM crop and the traditionally grown crop with respect to compositional characteristics has been carried out appropriately and that no statistically significant differences in the composition of the GM plant compared to its non-GM comparator have been identified.
We would like to remind that some Belgian experts have already expressed concerns about the fact that the compositional analysis is sufficient per se to drawn general conclusions concerning the safety of the whole GMO.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

- Allergenicity was tested by a bioinformatics approach. It means that allergenicity is identified versus known allergen proteins. Any new allergenic process will be missed.
- Allergenicity is tested by consultation of database. The date of this consultation and the size of the databases should be documented.

Comment 2

Simulated gastric fluid (SGF) was used to test the digestion of CP4 EPSPS proteins. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. It means that results on allergenicity testing provided in the dossier do not allow to conclude in the absence of risks.
The applicant is thus invited to provide any additional relevant data supporting its conclusion on allergenicity.

Comment 3

Assessment of allergenicity of the whole GM plant
The applicant did not evaluate the potential allergenicity of MON 88913 cottonseeds, compared to their traditional counterpart. The reviewer agrees, however, that cottonseed allergy is not a major issue, which means that a limited number of allergic sera can be available. Furthermore, no major

allergen of cottonseed has been defined. However, because the introduction of a new trait might influence the expression levels of other proteins of the host plant, it might be useful to evaluate the content of 2S storage protein and of vicillin, two known common seed allergens, in the MON 88913 cottonseed.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

A feeding study in channel catfish was conducted to evaluate the nutritional value of the MON88913 cottonseed. Energy metabolism in fish is similar to that in mammals and birds with two notable exceptions : (a) fish do not expend energy to maintain a body temperature different from that of their environment; and (b) the excretion of waste nitrogen requires less energy in fish than it does in homeothermic land animals (Anonymous, 1980). Fish may differ from mammals in its myofibre type: fish has more ßW fibres which may result in lower maintenance requirements.
In consequence, the applicant is invited to provide scientific justification on how far data obtained from studies in fish can be extrapolated to other species.
We would like also to know whether the nutritional value of the MON88913 cottonseed may be considered similar to that of the earlier GMO cottonseed MON1445.

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

Comments/Questions of the expert(s)

No comments.

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

Not applicable

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

No comments.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

In this chapter it is mentioned that the GMO cotton is of purely agronomic interest and presents negligible risk to the non-agricultural environments because the poor survival characteristics under most European conditions. How should we interpret “negligible” (are there data available?) and “poor survival characteristics” (this does not exclude a possibility of survival if the conditions were favourable for example in Southern European countries) ?
Indeed, Belgian experts already concluded that, in the context of the foreseen use of such GM cotton, accidental spillage of cotton seeds will be unavoidable (see i.e. advice of the Belgian Biosafety Advisory Council on dossier EFSA/GMO/NL/2005/13 - LLCotton25). If it occurs in southern Europe, it is likely that cotton would establish a feral population.
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- In case of establishment of feral population, what would be the impact on the receiving environment in terms of selective advantages or disadvantages?

D.9.3 Potential for gene transfer

No comments.

D.9.4 Interactions between the GM plant and target organism

Not applicable

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

No comments.

D.9.6 Effects on human health

No comments.

D.9.7 Effects on animal health

No comments.

D.9.8 Effects on biogeochemical processes

No comments.

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

No comments.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

No comments.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

No comments.

D.11.2 Interplay between environmental risk assessment and monitoring

No comments.

D.11.3 Case-specific GM plant monitoring

No comments.

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

No comments.

D.11.5 Reporting the results of monitoring

No comments.

References
see document BAC_2008_PT_637 in annex