Bioveiligheidsraad
Conseil de Biosécurité

23-09-2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2011_0898

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/BE/2010/79 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/BE/2010/79 was submitted by Monsanto on 17 May 2010 for the marketing of genetically modified soybean MON87701 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean MON87701 expresses the gene of the Cry1Ac protein that confers resistance against specific lepidopteran insects.

The application was officially acknowledged by EFSA on 11 June 2010. 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 genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). However, because this dossier repeats the data given in application EFSA/GMO/NL/2009/73 (Soybean MON87701 x MON89788) previously evaluated by seven Belgian experts, the same experts were asked to evaluate the parts of the dossier on MON87701 that have not been evaluated before. Three experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator and added to the comments already given for application EFSA/GMO/NL/2009/73. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 3 September 2010.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 6 July 2011 (EFSA Journal 2011; 9(7):2309)[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 1 August 2011 the opinion of EFSA 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. In addition, the complementary information regarding (i) molecular characterization and (ii) allergenicity testing sent by the applicant to EFSA in the course of the evaluation of the application was provided to the coordinator and to the experts who evaluated these aspects of the application. The comments

[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/2309.htm

Wetenschappelijk Instituut Volksgezondheid | Institut Scientifique de Santé Publique
Dienst Bioveiligheid en Biotechnologie | Service Biosécurité et Biotechnologie
Rue Juliette Wytsmanstraat 14 | B-1050 Brussels | Belgium
T + 32 2 642 52 11 | F + 32 2 642 52 92 | bac@wiv-isp.be | www.bio-council.be

formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the 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 compositional analysis as performed by the applicant, has not included the analysis of phosphatides in lecithin, as recommended by the OECD consensus document on compositional considerations for new varieties of soybean[4].

The Biosafety Advisory Council also considers that even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document, it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus document accordingly.

3.2. Assessment of toxicity

See point 3.1 and Conclusion.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed protein has been assessed as well as the allergenicity of the whole GM soybean. Due to technical limitations no exact identification of protein spots on a 2D immunoblot was achieved, making it impossible to look at specific immunodominant soybean allergens such as P34. But overall the the Biosafety Advisory Council is of the opinion that the total of the information provided by the applicant is sufficient and does not indicate the newly expressed protein to have allergic potential, nor does it indicate that the overall allergenicity of this soybean has been altered when compared to its conventional counterpart.

[3] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.
[4] OECD, 2001. Consensus Document on Compositional Considerations for New Varieties of soybean: Key Food and Feed Nutrients and Anti-Nutrients. ENV/JM/MONO(2001)15. http://www.oecd.org/dataoecd/15/60/46815135.pdf

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 answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council is of the opinion that the OECD recommendation regarding the comparative compositional analysis has not been completely followed.

Since the compositional analysis does not take away all concerns, the Biosafety Council looked at the animal trials. The absence of a sound explication of observed differences between the GM and the reference group, makes the Biosafety Council to give a negative advice regarding the health safety of the event.

Given the scope of the application of this insect resistant soybean MON87701 application (no cultivation in EU) and the fact that the establishment of volunteer plants would be unlikely (soybean cannot survive without human assistance and is not capable of surviving as a weed in Europe), the potential environmental release of MON 87701 is unlikely to pose any threat to the environment.

p.o. Dr. P. Herman

Prof. D. Reheul

President of the Belgian Biosafety Advisory Council

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/BE/2010/79 and *comments submitted on the EFSAnet (ref. BAC_2010_0852)*

Bioveiligheidsraad
Conseil de Biosécurité

3 September-2010

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2010_0852
Email. : bac@sbb.ihe.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2010/79
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 18 June 2010
Coordinator: René Custers
Experts for AP 73: Armand Christophe (UGent), Jacques Dommes (ULg), Leo Fiems (ILVO), Peter Smet (Consultant), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL), Johan Van Waes (ILVO)
Experts who evaluated the additional data submitted for AP 79: Leo Fiems (ILVO), Peter Smet (Consultant), Johan Van Waes (ILVO)
Domains of expertise of experts involved: Genome analysis, genetic engineering, molecular characterisation, transgene expression, human nutrition, biochemistry of food/feed, toxicology in vivo & in vitro, immunology, alimentary allergology, agronomy, agro-ecology, herbicide tolerance, soybean
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/BE/2010/79** concerns an application of the company **Monsanto** for the marketing authorisation of the genetically modified **soybean MON87701** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 June 2010.
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)

This dossier **EFSA/GMO/BE/2010/79** contains a lot of information that is similar to the recent application **EFSA/GMO/NL/2009/73 (Soybean MON87701 x MON89788)**. In order not to duplicate

any efforts the experts of the Biosafety Advisory Council were asked to evaluate the parts of the dossier on **MON87701** that have not been evaluated before. These are a re-evaluation data of the phenotype and morphology testing (now compared with conventional counterparts), and the results of a 90 day feeding trial. It only concerns parts **D.4.** and **D.7.8.4**. below.

As application EFSA/GMO/BE/2010/79 will be handled by EFSA separately the document repeats the comments received from the experts for application EFSA/GMO/NL/2009/73. Depending on their expertise, the experts who evaluated application EFSA/GMO/NL/2009/73 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). Comments made on elements that were already present in the EFSA/GMO/NL/2009/73 dossier have been repeated. 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 for both applications

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

There may be some concern about Cry1Ac protein: see further.
There is little chance that MON 87701 poses a safety risk as Cry1Ac is a non allergenic protein, and because it is heat labile and most soy products are processed. Therefore MON 87701 soybean and its by-products can be safely used.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No comments

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

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Under "3. Survivability – ability to form structures for survival or dormancy" it is mentioned that it is not likely that soybean seed would overwinter and germinate the following spring. My question is : are there data available of overwintering of seed of soybean for example in Southern Europe and in that case how were the volunteers destroyed?

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 3 (as given for application EFSA/GMO/NL/2009/73)

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

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 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No comments

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

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No comments

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

New data compared to application EFSA/GMO/NL/2009/79: The field phenotypic, agronomic and environmental interactions of US 2007 season (from CBI: Dunn et al., 2009)

Comments/Questions of the expert(s)

Comment 1 **(Van Waes)**

I agree with the conclusions of this study that there are no differences between MON 87701 and conventional soybean and commercially – available soybean varieties.

New data compared to application EFSA/GMO/NL/2009/79: The evaluation of seed germination and dormancy (from CBI: Dunn and Kendrick, 2009)

Comments/Questions of the expert(s)

Comment 1 **(Van Waes)**

I agree with the conclusions of this study.

Comment 2 **(Fiems)**

With regard to the dormancy and germination characteristics of MON 87701, mean values between MON 87701 and the control substance were not statistically different. Except its phyto-technical aspects, it is somewhat amazing that these parameters were presented, because the scope of the application for authorization of MON 87701 in the EU does not include the cultivation of MON 87701 varieties in the EU.

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

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Information adequate / no comments

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No comments

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 (as given for application EFSA/GMO/NL/2009/73)

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)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

1) The OECD guidelines for comparative assessment of soybean suggest to determine phosphatides in soybean matrices for human food (OECD 2001). This is not done in this application.
Recently, soy lecithin has been used for the cryopreservation of human sperm (Reed et al., 2009), to improve the productive and reproductive performance of hens, (Attia et al., 2009), and to change the fatty acid composition of milk (Gaby, 2009). Soy derived phospholipids are incorporated in infant formula and marketed as dietary supplements (e.g. Jorissen et al., 2002).

2*).* As pointed out in previous evaluation reports, it is suggested that saponins would be included in the compositional analysis of soybean.
Indeed, saponins are present in soy in relatively high quantities (Berhow et al., 2003) and although poorly absorbed in humans (Hu et al., 2004), they can cause bloat in ruminants (Van Haver et al., 2003) and induce enteritis in salmon (Knudsen et al., 2007). Soya sapogenols, obtained by hydrolysis of saponins, clearly have important biological effects (e.g. Zhang et al., 2008).

Remark SBB
For consistency with previous dossiers we suggest to transmit the comment concerning saponins preceded with the following sentence:
"Although the OECD consensus document on "Compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients" does not prescribe the analysis of saponins, one expert has suggested to include saponins in the compositional analysis."

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No questions

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

See 7.1

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

This section is well-documented. No further questions

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No questions

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1(as given for application EFSA/GMO/NL/2009/73)

From the calculations made on page 124 of Part I, it is clear that the concentration of Cry1Ac is not 0.002% in the seed as claimed in the text but in the seed protein fraction (as mentioned in the foodnote). Of course the conclusion that Cry1Ac in the seed is low remains true.

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

MON 87701 proteins did not show signs of toxicity when individually assessed in acute oral gavage studies in mice, so that we can conclude that the new proteins will not provoke toxicity problems.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Cry1Ac

The MON 87701-produced Cry1Ac protein has 100% amino acid identity with the Bollgard MON 531 cotton expressed Cry1Ac protein, except for the four additional amino acids at the N-terminus of the MON 87701-produced protein. The potential for toxicity of the cry1Ac gene expression product was assessed (Annex 1 of the Technical Dossier part 1). The identity of the plant-produced Cry1Ac protein was verified by: N-terminal sequencing, proteolytic peptide mapping followed by MALDI-TOF MS analysis, Western Blot analysis, SDS-PAGE, and an insect growth inhibition assay. The equivalence between the *E. coli*-produced (and for toxicity testing used) Cry1Ac protein and the MON 87701-produced Cry1Ac protein was established by: SDS-PAGE, Western Blot analysis, glycosylation analysis, and an insect growth bioassay.

A detailed bioinformatics analysis demonstrated that the Cry1Ac protein does not show structural similarity to known toxins or other biologically active proteins that could cause adverse effects. The acute oral toxicity study with CD-1 mice demonstrated that the Cry1Ac protein is not acutely toxic and

does not cause any adverse effects. No treatment-related effects were observed on survival, clinical observation, body weight gain, food consumption or gross pathology (NOAEL ♂ = 1460 mg/kg bw, NOAEL ♀ = 1290 mg/kg bw). As such, large MOE's have been demonstrated. Additionally, the rapid digestibility of the full-length Cry1Ac protein in simulated digestive fluids (SGF and SIF) was demonstrated (The transiently stable protein fragment ~4 kDa which was observed at the 30s time point in SGF, was digested in less than 1 min by SIF).
In conclusion: the extensive data set indicates that the MON 87701-produced Cry1Ac protein is safe for food/feed use.
No further comments.

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Mean concentrations in different MON87701 tissues of Cry1Ac protein have been determined and expressed on a dry weight basis. In terms of food and feed safety assessment of MON 87701 seed and forage are the most relevant tissues.
Based on these results, an estimated protein intake was calculated:

- Cry1Ac: general population = 0.0239 mg/kg/dag; children < 6 years old = 0.0439 mg/kg/dag

a) Degradation of the Cry1Ac protein in simulated gastric fluid (Goertz et al., 2008).

The results of the study demonstrated that greater than 99% of the full-length Cry1Ac protein was digested in SGF within 30 s when analyzed using Colloidal Brilliant Blue G stained SDS-PAGE, and at least 95% of the full-length Cry1Ac protein was digested within 30 s when analyzed by western blot with a Cry1Ac-specific antibody. At least 95% of the full-length Cry1Ac protein was digested, as expected, to the trypsin-resistant core (~55 kDa) within 5 min during incubation in SIF alone. A transiently stable protein fragment migrating at ~4 kDa was observed during SGF digestion when analyzed using a Colloidal Brilliant Blue G stained polyacrylamide gel, but neither this fragment, nor any other immunoreactive peptides were detected by western blot analysis. The identity of the ~4 kDa fragment was determined by N-terminal sequencing to be a mixture of two degradation peptides from the Cry1Ac protein. The two identified peptides matched Cry1Ac sequence starting at amino acid positions 415 and 882. When the Cry1Ac protein was subjected to the sequential enzymatic digestion, i.e. digestion in SGF followed by a short digestion in SIF, the ~4 kDa fragment degraded in less than 1 min upon exposure to SIF.

b) Degradation of the Cry1Ac protein in simulated intestinal fluid (Goertz et al., 2008).

See above.

c) Cry1Ac: Acute Oral Toxicity Study inMice ().

Cry1Ac protein was administered by oral gavage to 10 male and 10 female CD-1 mice at a total dose of **1290 mg of protein /kg body wt**, administered in two doses of 33.3 ml/kg of body weight, separated by about 4 hours). Additional groups of 10 male and 10 female mice were administered a comparable dose of bovine serum albumin (BSA) (1280 mg of protein /kg body wt) to serve as a protein control.
There were no treatment-related effects of Cry1Ac on survival, clinical observations, body weight gain, food consumption or gross pathology. A statistically significant reduction in body weight gain was observed in males but not in females dosed with 1290 mg/kg Cry1Ac relative to BSA-treated controls,

however, this result was considered equivocal because at least one male in the study experienced an interruption in water supply. In order to further investigate this possible effect on body weight, an additional group of 10 **male** CD-1 mice (and BSA controls) was dosed with Cry1Ac by oral gavage at a total dose of **1460 mg/kg body wt** (two equal doses four hours apart). There was no effect on body weight in males dosed with 1460 mg/kg Cry1Ac.

d) Cry1Ac: Assessment of Amino Acid Sequence Homology with Known Toxins (**From CBI:** Silvanovich and Tu, 2009a)

The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the Cry1Ac protein and any known toxic or other biologically active proteins that would be harmful to human or animal health. Additionally, results of the alignments with the entire T-DNA to the TOX_2009 database revealed no relatedness with known toxins and other relevant biologically active proteins

Conclusion concerning the testing of new protein: Cry1Ac is readily degraded in SIF and SGF. No toxic effects were observed during acute testing. NOAELs were determined tot be 1290 mg/kg for Cry1Ac.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No further comments. No testing of any constituent other than the introduced protein is indicated.

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

No questions

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Compositional analyses were performed on forage and seed collected from MON 87701 at 2 different field trails with each five field sites: US 2007 season (**From CBI:** Berman et al., 2008a,b), and Argentina 2007-2008 season (**From CBI:** Berman et al., 2009a,b).

Argentina 2007-2008
Test: MON 87701 (R9 generation)
Control : conventional A5547, MON 89788 (not used)
References : 20 commercial conventional soybean varieties

Analysis of the combined-site data set:
Forage:
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 and A5547: tryptophan, 18:3 linolenic acid, **vitamin E**, stachyose

Analysis of the individual-site data set (more than 1 site):
Forage:
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 and A5547: tryptophan (2 sites), 18:3 linolenic acid (3 sites), **vitamin E (5 sites)**

However, mean values of MON 87701 for all these components fell within the 99% tolerance interval established from the commercial reference soybean varieties.

US 2007
Test: MON 87701 (R8 generation)
Control : conventional A5547, MON 89788 (not used)
References : 20 commercial conventional soybean varieties (the same varieties as grown in the Argentina 2007-2008 field trial except for 1 variety)

Analysis of the combined-site data set:
Forage:
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 and A5547: proteine, alanine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, valine, 22:0 behenic acid, carbohydrates, **vitamin E**, trypsin inhibitor, daidzein

Analysis of the individual-site data set (more than 1 site):
Forage:
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 and A5547: histidine (2 sites), 22:0 behenic acid (2 sites), **vitamin E (4 sites)**, daidzein (2 sites), stachyose (2 sites)

In conclusion:
No consistent alteration in the level of the studied components (except for vitamin E) was found between sites/growing seasons/field trials. Furthermore, the differences were generally small (except for vitamin E) and fell (vitamin E included) within the interval of natural variation calculated from the

occurrence of these constituents in conventional soybean varieties. The analyte values were also comparable to values published in the scientific literature and reported in ILSI.
Additional comment for vitamin E: No dietary impact is expected as the vitamin E levels are comparable to the values reported in ILSI. This was confirmed with the 42-day feeding study in broilers.
It can be concluded that the forage and seed of MON87701 are compositionally equivalent to conventional soybean forage and seed.

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Saponins were not included in the analysis (see comment under D.7.1).

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

<u>42-day feeding study in broilers.</u>
A 42-day feeding study with broilers was conducted with diets containing soybean meal from the test soybeans MON 87701, a conventional control, and six conventional soybean varieties. The test and control soybeans were grown during the US 2007 trial; the six additional varieties were grown at other locations. Chemical and nutrient analyses were performed prior to initiating the study.
There were no biologically relevant differences in broiler performance, carcass yields or meat composition between broilers fed diets containing soybean meal produced form MON 87701 and those broilers fed diets containing the conventional control.
In conclusion, this study did not indicate any toxic effects and any unanticipated or pleiotropic effects.

New data compared to application EFSA/GMO/NL/2009/79: 90-day oral feeding study with MON87701 (from CBI: WIL-50352, 2009)

Comments/Questions of the expert(s)

Comment 1 **(Van Waes)**

No remarks

Comment 2 **(Fiems)**

Although not specifically related to the toxicity study with rats, but for risk assessment and safety reasons, it seems more appropriate to take a maximum intake into account. The applicant refers to NRC studies to assume a crude protein intake in chickens, pigs and dairy cows. However, from a meta-analysis Ipharraguerre and Clark (2005) reported a maximum daily nitrogen intake of 855g, corresponding to 5344g crude protein. In case a of cow of 680 kg (NRC, 2001), this means a crude protein intake of 7.86g, or 30% more than in the dossier. This may result in 0.000164 instead of 0.000126 g Cry1Ac per kg body weight daily for lactating dairy cows, which is still rather low.

Comment 3 **(Smet)**

Different parameters were examined. Although several of them were significant different compared to the control group, to my point of view, these differences aren't related to the administration of MON 87701, because:

1. the initial as well as the repeat study do not lead to the same conclusions and,
2. the absence of dose-response relationships.

For the moment no further testing is needed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

1) It has been reported that IgE antibodies from soybean-sensitive patients recognise more than 15 soybean proteins (Krishnan et al., 2009). Over-expression of proteins, some of which may be allergenic, is a possibility in transformed plants. Thus the potential for increasing the endogenous allergenicity of an already allergic crop has to be considered. This has been evaluated by measuring the reactivity of protein extracts of MON87701 with sera of allergic persons.

2) Soy proteins are incorporated in some infant formulas (D'Auria et al.; 2005). Gastric proteolysis is limited in infants (Hamosh; 1996) and soy products may contain protein P34 which is the immunodominant soybean allergen (Wilson et al.; 2008). Thus it may be of value to determine whether this allergen is increased or not in MON87701.

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

There may be some controversy around the safety of Cry1Ac. Vázquez-Padrón et al. (2000) indicated that CrylAc was a potent systemic and mucosal immunogen and its protoxin (pCry1Ac) binded to the mucosal surface of the mouse small intestine by immunohistochemical test. Moreover, this protein induced in situ temporal changes in the electrophysiological properties of the mouse jejunum. The above data indicated a possible interaction in vivo of Cry proteins with the animal bowel, which could induce changes in the physiological status of the intestine. But other researchers concluded that GMO (Bt-Cry1Ac gene) cottonseed meal had no deleterious effect on growth performance, blood biochemicals and various carcass characteristics of growing broiler chickens (Elangovan et al., 2006). The GM cottonseed expressing Cry1F, Cry1Ac and PAT proteins had no adverse effects in 90 days of feeding test (Dryzga et al., 2007). Wu et al. (2009) concluded that there is a reasonable certainty of no harm resulting from the inclusion of the Cry1Ab/Ac protein in human food or animal feed.

The rapid digestibility in simulated digestive fluids is not a guarantee for safety. 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 and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.

Additional comment from SBB

To be consistent with comments previously transmitted in the frame of the evaluation of dossier RX-RX-MON531(cotton) the SBB proposes to add the following comment:

If Cry1Ac is not likely to be an allergen itself, it should be emphasized that Cry1Ac has been proposed as an adjuvant for vaccines (Esquivel-Pérez and Moreno-Fierros, 2005; Moreno-Fierros et al., 2003; Vásquez et al., 1999; Vásquez-Padrón et al., 1999; Verdin-Terán al. 2009), which means that this protein is able to enhance the immune responses against antigens that are co-administered. This is not uncommon for a bacterial protein. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. It is not known whether the presence of Cry1Ac might elicit sensitization against the other plant proteins upon ingestion. It might be relevant to study in mice the immune responses against soya proteins when the animals are fed Soybean MON87701.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No questions

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Only the content of vitamin E is presented and discussed. Why are other vitamins not presented and discussed?

Remark from the coordinator

Vitamins are not required to analyse according to the OECD consensus document.

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)

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 (as given for application EFSA/GMO/NL/2009/73)

No questions

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Based on the studies of Elangovan et al. (2006), Dryzga et al. (2007) and Wu et al. (2009), we may conclude that there is a reasonable certainty of no harm resulting from the inclusion of the Cry1Ac protein in human food or animal feed.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

No questions

Comment 2 (as given for application EFSA/GMO/NL/2009/73)

Based on the studies of Elangovan et al. (2006), Dryzga et al. (2007) and Wu et al. (2009), we may conclude that there is a reasonable certainty of no harm resulting from the inclusion of the Cry1Ac protein in human food or animal feed.

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 (as given for application EFSA/GMO/NL/2009/73)

In this paragraph it is mentioned again that the scope of application does not include cultivation of soybean 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-soybean be incorporated in normal VCU trials, for example treated with specific herbicides for

soybean and will the agronomical value be the same as tested in trials, where the herbicide glyphosate, for which the variety is tolerant, is used?

Remark from the coordinator
I would claim that the agronomical value is different because of the fact that the glyphosate spraying can be less precise in timing than conventional herbicides. And besides the CryIAc protein will give an agronomical difference.
The remarks made are not relevant for the safety assessment.

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 (as given for application EFSA/GMO/NL/2009/73)

The proposed environmental monitoring plan is OK

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1 (as given for application EFSA/GMO/NL/2009/73)

Based on the scope of application (no cultivation) I can agree with the remark of this chapter.

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

Attia YA, Houssein AS, Tag El-Dine et al. (2009). Improving productive and reproductive performance of dual-purpose crossbred hens in the tropics by lecithin supplementation. Trop Anim Health Prod 41:461-475.

Bannon, G., Fu, T.J., Kimber, I., Hinton, D.M. 2003. Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124.

D'Auria E, Agostini C et al. (2005). Proteomic evaluation of milk from different mammalian species as a substitute for breast milk. Acta Paed 94:1708-1713.

Dryzga, M.D., Yano, B.L., Andrus, A.K., Mattsson, J.L., 2007. Evaluation of the safety and nutritional equivalence of a genetically modified cottonseed meal in a 90-day dietary toxicity study in rats. Food Chem. Toxicol. 45: 1994–2004

Elangovan, A.V., Tyagi, P.K., Shrivastav, A.K., Tyagi, P.K., Mandal, A.B., 2006. GMO (Bt-Cry1Ac gene) cottonseed meal is similar to non-GMO low free gossypol cottonseed meal for growth performance of broiler chickens. Anim. Feed. Sci. Technol. 129: 252-263.

Esquivel-Pérez R and Moreno-Fierros L.(2005) Mucosal and systemic adjuvant effects of cholera toxin and Cry1Ac protoxin on the specific antibody response to HIV-1 C4/V3 peptides are different and depend on the antigen co-administered. Viral Immunol. 18(4):695-708.

Gaby AR (2009). Nutritional approaches to prevention and treatment of gallstones. Altern Med Rev 14: 258-267.

Hamosh M (1996). Digestion in the newborn. Clin Perinatol 23:191-209.

Herman, R.A., Storer, N.P., Gao, Y. 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154-1157.

Hu J, Reddy MB, Hendrich S, Murphy PA (2004). Soyasaponin I and sapongenol B have limited absorption by Caco-2 intestinal cells and limited bioavailability in women. J Nutr 134: 1867-1873.

Ipharraguerre, I.R., Clark, J.H. 2005. Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J. Dairy Sci. 88: E22–E37.

Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ (2002). Safety of soy-derived phosphatidylserine in elderly people. Nutr Neurosci 5:337-343.

Knudsen D, Jutfelt F, Sundh H, Sundell K, Koppe W, Fokiar H (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (Salmo salar L.). Br J Nutr. 100:120-129.

Krishnan HB, Kim WS, Jang S, Kerly MS (2009). All three subunits of soybean beta-conglycinin are potential food allergens. J Agric Food Chem 57:938-9430.

Moreno-Fierros L. et al. (2003) Intranasal Cry1Ac Protoxin is an Effective Mucosal and Systemic Carrier and Adjuvant of Streptococcus pneumoniae Polysaccharides in Mice. Scandinavian Journal of Immunology 57 (1) , 45–55

NRC, 2001. Nutrient requirements of dairy cattle (Seventh revised edition). National Academy Press, Washington, D.C., 381 pp.

OECD 2001. Consensus document on compositional considerations for new varieties of soybean : Key food and feed nutrients and anti-nutrients. ENV/JM/MONO (2001) 15 page 20.

Reed ML, Ezeh PC, Hamic A, Thompson DJ, Caperton CL, 2009. Soy lecithin replaces egg yolk for cryopreservation of human sperm without adversely affecting postthaw motility, morphology, sperm DNA integrity, or sperm binding to hyaluronate. Fertil Steril 92:1787-1790.

Spök, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, W., Valenta, R. 2005. Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int. Arch. Allergy Immunol. 137: 167-180.

Vásquez et al. (1999) Bacillus thuringiensis Cry1Ac Protoxin is a Potent Systemic and Mucosal Adjuvant. Scand J Immunol, 49:578-84

Vásquez- Padrón, R.I., Moreno- Fierros, L. Neri-Bazán, L, de la Riva, G.A. & López-Revilla, R. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sciences 64: 1897-1912;

Vázquez-Padrón, R.I., Moreno-Fierros, L., Neri-Bazán, L., Martínez-Gil, A.F., de-la-Riva1, G.A., López-Revilla, R., (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz. J. Med. Biol. Res. 33: 147–155.

Verdin-Terán SL, Vilches-Flores A, Moreno-Fierros L. Immunization with Cry1Ac from Bacillus thuringiensis increases intestinal IgG response and induces the expression of FcRn in the intestinal epithelium of adult mice. Scand J Immunol. 2009 Dec;70(6):596-607.

Wilson S, Martinez-Villaluenga C, Demeija EG (2008). Purification, thermal stability, and antigenecity of the immunodominant soybean allergen P34 in soy cultivars, ingredients and products. J Food Sci 73: T104-114.

Wu, W., Cao, S., He, X., Luo, Y., Guo, X., Yuan Y., Huang, K. 2009. Safety assessment of Cry1Ab/Ac fusion protein. Food Chem. Toxicol. 47: 1459-1465.

Zhang W, Popovich DG. (2008). Effect of soyasapogenol A and soyasapogenol B concentrated extracts on HEP-G2 cellproliferation and apoptosis. J Agric Food Chem 56:2603-2608.