Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0444

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2009/73 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2009/73was submitted by Monsanto on 27 August 2009 for the marketing of genetically modified soybean MON87701 x MON89788 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean MON87701 x MON89788 expresses the gene of the Cry1Ac protein that confers resistance against specific lepidopteran insects and the gene of the CP4 EPSPS protein conferring tolerance to glyphosate-based herbicides.

The application was officially acknowledged by EFSA on 8 December 2009. 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). Seven 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 5 March 2010.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 26 January 2012 (EFSA Journal 2012; 10(2):2560[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 16 February 2012 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. The comments 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.
In addition, the scientific evaluations of the single events, namely soybean line MON87701 (EFSA/GMO/BE/2010/79), and line MON89788 (EFSA/GMO/NL/2006/36) are taken into account in this advice. The Biosafety Council formulated a positive advice for line MON89788. For line MON87701, because OECD recommendation regarding the comparative

[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/2560.htm

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compositional analysis had not been completely followed, the Biosafety Advisory Council gave a negative advice for this GM soybean[3].
The two single soybean events are authorised by the European Commission for food and feed uses[4].

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment in the European Union in the context of the intended use[5].

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 notifier, has not included the analysis of phosphatides in lecithin, as recommended by the OECD consensus document on compositional considerations for new varieties of soybean[6].

The Biosafety Advisory Council also considers that, although not required by the OECD, 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

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 potential allergenicity of the newly expressed proteins has been assessed as well as the allergenicity of the whole GM soybean. It is unlikely that the newly expressed proteins change the allergenicity of the whole crop.

[3] Advice of BAC on soybean line MON87701: BAC_2011_0898; Advice of BAC on soybean line MON89788: BAC_2008_813;
[4]See GMO register : < http://ec.europa.eu/food/dyna/gm_register/index_en.cfm>
[5] 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.
[6]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

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 soybean with its non-GM counterpart and conventional soybean varieties.

4. Monitoring

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

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the 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 applicant did not follow the OECD recommendation on compositional considerations for new varieties of soybean and did not argue why not. Based on the currently available information, the Biosafety Advisory Council does not give an advice on the health safety of soybean MON87701 x MON89788.

Given the scope of the application of this insect resistant and herbicide tolerant soybean MON87701 x MON89788 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 dispersion of MON87701 x MON89788 is unlikely to pose any threat to the European environment.

p.o. Ph. Heyrman

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex I: Additional remark from the Biosafety Advisory Council
Annex II: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2009/73 and *comments submitted on the EFSAnet (ref. BAC_2010_0211)*

Annex I : Additional remark from the Biosafety Advisory Council

Next to the biosafety assessment of imported soybean in the European Union, the Biosafety Advisory Council is concerned about the health hazards for the people in some countries growing glyphosate tolerant soybeans. The Council will prepare a letter expressing these concerns to be sent to the Competent Authorities in search for a competent forum where this topic can be discussed.

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05-03-2010

Secretariaat
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Compilation of comments of experts in charge of evaluating the application EFSA/ GMO/NL/2009/73
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 11 December 2009
Coordinator: René Custers
Experts: Armand Christophe (UGent), Jacques Dommes (ULg), Leo Fiems (ILVO), Peter Smet (Consultant), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL), 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/NL/2009/73** concerns an application of the company **Monsanto** for the marketing of the genetically modified **Soybean MON87701 x MON89788** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 8 December 2009.
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

MON 87701 × MON 89788 is obtained by traditional breeding of two inbred lines: MON 87701 and MON 89788. The introduction of CP4 EPSPS into the soybean genome resulted in MON 89788. EFSA (2005) adopted a favourable opinion which resulted in the approval of this soybean. Furthermore, Ash et al. (2003) reported that the digestive process of the laying hen effectively broke down the CP4 EPSPS protein from the soybean meal.

The introduction of Cry1Ac into the soybean genome resulted in MON 87701. 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 × MON 89788 soybeans and its by-products can be safely used.

P.96-105 of Part I of the Dossier: According to the title, Table 22 presents the compositional analysis of **maize** forage and grain collected from MON 87701: it is assumed that not maize, but data from soybean are presented?

Comment 2

According to the dossier the scope of application does not include the authorization for the cultivation of MON 87701 x MON 89788 soybean products in the EU. It can however be worthful 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.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Information adequate / no comments

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

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

Information adequate / no comments

Comment 3

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

Information adequate / no comments

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

Information adequate / no comments

Comment 2

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

The mean level of Cry1Ac in seed seems to be significantly different in seeds from MON87701 compared to seed from MON87701x MON89788 (based on the reported means, standard deviations and number of replicates in Table 6, Part I, Technical Dossier page 39, I estimated a $p < 0.0001$). If so, why were p values not reported and more importantly, how can this be explained?
(interaction between the 2 newly introduced genes, difference in insert number,…?)

Comment 2

Information adequate / no comments

Comment 2

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)

Comment 1

Information adequate / no comments

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

Information adequate / no comments

Comment 2

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

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

In this chapter it is mentioned that MON 87701 x MON 89788 soybean was compared to other commercial conventional soybean varieties. What does it mean? The MON 87701 x MON 89788 soybean 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 87701 x MON 89788 soybean 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?

Remarks from the coordinator

- *The GM plant should be compared to a comparator with a genetic background that is as close as possible, plus a number of conventional varieties. And three regimes should be applied: GM + glyphosate, GM + conventional herbicides and conventional + conventional herbicides.*
- *The EFSA guidelines do not require comparison with other GM varieties.*

Comment 2

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).

Questions: Table 22 in Technical Dossier, Part 1,

a) Pages 95-104, in heading: Compositional analysis of **maize** forage and seed: printing error??
b) Page 100: I assume that the reported values for fatty acids in the columns representing MON 89788 /Arg(entine study) and control Arg(entine study) are wrong. This makes interpretation of the stack results not possible.

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."

Comment 3

All mean values of MON 87701 × MON 89788 observed to be significantly different from the control values were within the 99% tolerance interval established from the conventional commercial reference soybean varieties. These values were also comparable to the ranges published in the scientific literature and the International Life Sciences Institute (ILSI) Crop Composition Database.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

See 7.1

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

It is mentioned that from field trials MON 87701 x MON 89788 soybean is equivalent to the conventional soybean, except for the introduced traits.
From this information we can conclude that there is no significant difference in the agronomical value between the MON 87701 x MON 89788 soybean and the conventional type. Is this conclusion correct?

Remark from the coordinator

The equivalence is only applicable to the composition of the soybean, but has nothing to do with its agronomical value.

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

This section is well-documented. No further questions

Comment 2

No questions

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

MON 87701 x MON 89788 is produced by traditional breeding of MON 87701 and MON 89788.
MON 89788 was approved in 2008 for import, processing, and food and feed use in the EU (Commission Decision 2008/933/EC).
The safety of the MON 87701 variety has not been assessed yet.

Comment2

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 3

MON 87701 × MON 89788 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

CP4 EPSPS

The absence of toxic potential associated to the CP4 EPSPS protein has been previously established by EFSA. EFSA delivered a positive opinion on the safety of the introduced CP4 EPSPS protein expressed in MON 89788. Additionally, an updated bioinformatics analysis demonstrated that the CP4 EPSPS protein does not show structural similarity to known toxins or other biologically active proteins that could cause adverse effects.
No further comments on the safety of the CP4 EPSPS protein.

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

Safety aspects of the individual newly expressed proteins are amply described (e.g. Part I, page 121) . Only a broiler chicken feeding study is performed to investigate if combined presence of the newly expressed proteins in feed poses safety concerns. **Regulatory question:** Would it be indicated in case of stacked events (or more general when more than one protein is expressed by the transformation event) to investigate possible interactions between the newly expressed proteins in other systems (e.g. a 90-day toxicity study in rodents…?).

Remark from the coordinator
This would only be necessary when there is a trigger to do so. There is no reason to suspect any possible interaction between the Cry protein and the EPSPS protein. Moreover so because the soybean already possesses a EPSPS protein naturally (although slightly different from the CP4 one).

Remark SBB
Decision of the Council in June 2009: "Concerning the evaluation of the toxicity of transgenic proteins in stacked events, in the advices of the Council it will not be requested to evaluate their interaction when these interactions are very unlikely (for example when the proteins have different receptors).
According to De Schrijver et al. (2007) only if uncertainty remains or if differences (change in natural amounts of toxins revealed by compositional analysis) are indeed confirmed, overall toxicity testing of the GM stacked event may be considered."

Comment 3

Mean concentrations in different MON87701 x MON89788 tissues of both Cry1Ac and CP4 EPSPS protein have been determined and expressed on a dry weight basis. In terms of food and feed safety assessment of MON 87701 × MON 89788, 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
- CP4 EPSPS : general population = 0.4848 mg/kg/dag; children < 6 years old = 0.8880 mg/kg/dag

a) Degradation of the CP4 EPSPS protein in simulated gastric fluid ().

CP4 EPSPS was shown to be rapidly degraded in both an *in vitro* simulated gastric fluid (SGF) digestion model and an *in vitro* simulated intestinal fluid (SIF) model (Leach *et al.*, 2002; Ream *et al.*, 1993). The data demonstrate a half life for CP4 EPSPS of less than 15 seconds in the simulated gastric system and less than 10 minutes in the intestinal system, based on western blot analysis of the digested products.

b) Degradation of the CP4 EPSPS protein in simulated intestinal fluid ().

See above.

c) CP4 EPSPS: Acute Oral Toxicity Study inMice ().

There were no treatment-related adverse effects in mice administered the CP4 EPSPS protein by oral gavage at dosages up to **572 mg/kg**.
There were no statistically significant ($p \leq 0.05$) differences in body weight, cumulative body weight, or food consumption between the vehicle and bovine serum albumin protein control groups and CP4 EPSPS protein-treated groups.

d) CP4 EPSPS: Assessment of Amino Acid Sequence Homology with Known Toxins ()

Results of these most recent searches indicate that none of the putative flank polypeptides share significant sequence similarity with sequences in the AD_2009 and TOX_2009 databases.
Furthermore, no eight amino acid matches were shared between the putative flank polypeptides and sequences in the AD_2009 database. When combined, these data confirm the previously reported conclusion (McClain and Silvanovich, 2006) that no meaningful sequence or inferred structural similarity exists between the putative flank polypeptides and allergenic and toxic proteins.

e) 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.

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

See above.

g) 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.

h) 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 proteins: Both Cry1Ac and CP4 EPSPS are readily degraded in SIF and SGF. No toxic effects were observed during acute testing. NOAELs were determined tot be 572 mg/kg for CP4 EPSPS and 1290 mg/kg for Cry1Ac.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

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

Comment 2

No questions

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Compositional analyses were performed on forage and seed collected from MON 87701 and MON 87701 x MON 89788 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 x MON 89788, MON 87701 (R9 generation)
Control : conventional A5547, MON 89788 (not used)
References : 20 commercial conventional soybean varieties
MON 87701 x MON 89788 and MON 89788 were glyphosate-treated (**From CBI:** Mulesky, 2009).

Analysis of the combined-site data set:
Forage:
Significant differences between MON 87701 x MON 89788 and A5547: ADF
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 x MON 89788 and A5547: glutamic acid, leucine, 18:0 stearic acid, 18:2 linoleic acid, 20:0 arachidic acid, ash, **vitamin E**, stachyose, daidzein, genistein
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 x MON 89788 and A5547: 0
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 x MON 89788 and A5547: 18:0 stearic acid (4 sites), 18:2 linoleic acid (3 sites), ash (2 sites), **vitamin E (3 sites)**, daidzein (2 sites), genistein (2 sites)
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 x MON 89788 and NON 87701 for all these components fell within the 99% tolerance interval established from the commercial reference soybean varieties.

US 2007
Test: MON 87701 x MON 89788, 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)

MON 87701 x MON 89788, MON 89788, and 3 commercial varieties (?) were glyphosate-treated (Armstrong, 2008).

Analysis of the combined-site data set:
Forage:
Significant differences between MON 87701 x MON 89788 and A5547: protein
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 x MON 89788 and A5547: alanine , aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, serine, threonine, valine, 16:0 palmitic acid, 18:0 stearic acid, 18:3 linolenic acid, 20:0 arachidic acid, protein, lectin, daidzein, genistein,
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 x MON 89788 and A5547: 0
Significant differences between MON 87701 and A5547: 0

Seed:
Significant differences between MON 87701 x MON 89788 and A5547: arginine (2 sites), histidine (2 sites), isoleucine (2 sites), leucine (2 sites), serine (2 sites), valine (2 sites), 14:0 myristic acid (2 sites), 16:0 palmitic acid (3 sites), 16:1 palmitoleic acid (2 sites), 18:0 stearic acid (4 sites), 18:3 linolenic acid (2 sites), 20:0 arachidic acid (4 sites), daidzein (3 sites), genistein (2 sites), glycitein (2 sites), **vitamin E (3 sites)**, stachyose (2 sites)
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 MON 87701 x MON 89788 and MON87701 are compositionally equivalent to conventional soybean forage and seed.

Comment 2

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

42-day feeding study in broilers.
A 42-day feeding study with broilers was conducted with diets containing soybean meal from the test soybeans MON 87701 and MON 87701 x MON 89788, 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 or MON 87701 x MON 89788 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.

90-day oral toxicity study in rodents.
Not performed for MON 87701 and MON 87701 x MON 89788.

No additional 90-day oral toxicity study in rats is requested. The thorough evaluation of the safety of the Cry1Ac and CP4 EPSPS proteins, the compositional analyses, and the broiler feeding study did not indicate safety concerns for MON 87701 and MON 87701 x MON 89788.

No further comments.

Comment 2

No questions

Comment 3

a) 42-day feeding study in broiler chickens ().
There were no biologically relevant differences in broiler performance, carcass yield or meat composition between broilers fed diets containing SBM produced from MON 87701 × MON 89788 and those fed diets containing SBM (soybean meal) produced from conventional control soybean.

b) 90-Day rat feeding study ().
Not performed. At this moment, no further testing is needed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

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 and MON89788 separately with sera of allergic persons.
Regulatory question: is it sufficient to demonstate that the endogenous allergenicity of the genetically modified parental lines is probably not increased or must this also be investigated for offspring obtained by breeding of these lines?

Note from the SBB
"If it has been demonstrated that the inserts and junction regions are maintained and that the introduced traits or newly expressed chimaeric proteins present in the single events are not demonstrated to act as allergens or toxins in the GM paranteral inbred lines, it is not relevant to demonstrate this again in the GM stacked event" (De Schrijver et al., 2007)

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 MON87701xMON89788 (and in all stacked and transformed stacked soybean varieties in general).

Comment 2

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 x MON8978.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

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

No questions

Comment 2

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. According to EFSA (2005) the safety of CP4 EPSPS protein has previously been assessed in the single events, for which positive opinions were issued.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

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. According to EFSA (2005) the safety of CP4 EPSPS protein has previously been assessed in the single events, for which positive opinions were issued.

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 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

The proposed environmental monitoring plan is OK

Comment 2

There is a recent evolution of glyphosate resistance in weed populations. Gaines et al. (2010) reported a glyphosate resistance in Amaranthus palmeri, due to many-fold amplification of the EPSPS gene on multiple chromosomes. This occurrence of gene amplification as an herbicide resistance mechanism was observed in a naturally occurring weed population. This occurrence of such gene amplification as an herbicide resistance mechanism in a naturally occurring weed population is particularly significant because it could threaten the sustainable use of glyphosate-resistant crop technology. Even if the culture of MON 87701 x MON 89788 soybean is not relevant for the EU, special attention should be paid to the environmental consequences of the introduction of more genetically modified crops based on the insertion of EPSPS into the genome.

Remark from the coordinator
Indeed the resistance development issue is important for dossiers that involve cultivation. But it is not relevant for this dossier.

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 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)

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