Biosafety Advisory Council

19-03-2008

Secretariat

O./ref.: WIV-ISP/BAC/2008_685[1]
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Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/NL/2005/18** of Bayer CropScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2005/18 was submitted by Bayer CropScience in July 2005 for the marketing (import and processing) of the glufosinate-tolerant genetically modified soybean A2704-12 for food and feed applications under Regulation (EC) No. 1829/2003[2]. It was officially acknowledged by EFSA on 10 February 2006.

On the same date EFSA started the 3 months formal consultation 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 (GMOs) being part of the products).

In the frame of this consultation, the Belgian Biosafety Advisory Council, 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 Biosafety Advisory Council and the Division of Biosafety and Biotechnology. Three experts answered positively to this request, and formulated a number of comments to the dossier synthesised by the coordinator. For an overview of all the comments and for comments actually placed on the EFSAnet on 9 May 2006 see Annex I.

The opinion of EFSA's scientific panel on GMOs was adopted on 3 July 2007 (The EFSA Journal, 2007, 524, 1-22)[3]

[1] Replaces document BAC_2008_SC_635 of 23 January 2008

[2] 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)

[3] see: http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/1062.html

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On 26 July 2007 the opinion of EFSA was forwarded to the Belgian experts. The experts were invited to give comments and to react 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 the EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

Scientific evaluation

According to the Biosafety Advisory Council, no major risks were identified neither concerning the molecular characterisation, the toxicity, the allergenicity nor the environment[4].

Following the comments submitted by the Belgian experts, the Biosafety Advisory Council considers that even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document[5], it lacks the analysis on dietary fibre while this concept is widely accepted in human food studies.

Important comments

From a scientific point of view, the dossier lacks clarity and transparency, which induces confusion in interpreting the results. In particular the applicant confounds results between non transgenic plants, transgenic plants that were not treated with the herbicide and transgenic plants that were treated with the herbicide. As a consequence it becomes difficult to assess the interaction between the GMO and the herbicide.
This remark is based on data presented for the compositional analysis. The applicant presents data for ***whole beans*** of non-transgenic plants and of transgenic plants either treated or not with the herbicide. So this analysis is complete. Analysing the ***toasted meal***[6], the applicant fails to present data of non-herbicide treated transgenic plants. However, in crop production systems transgenic plants are herbicide treated.

Given there is no difference in composition in the ***whole beans***, some members of the Biosafety Advisory Council consider that this failure in data presentation does not present a problem to assess the effect on health, while some other members do not agree with this interpretation.

[4] As the application doesn't imply a cultivation of the plant in EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

[5] 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.olis.oecd.org/olis/2001doc.nsf/c5ce8ffa41835d64c125685d005300b0/

[6] The meal is what remains after the fat extraction from the whole beans

The Biosafety Advisory Council would also like to make a comment on the opinion of the EFSA GMO panel. On page 15, Point 5.2.1.1, last §, EFSA states that "Therefore the GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean A2704-12 in Europe will not be different to that of conventional varieties".
This sentence sounds too broad and opens the door for future requests of cultivation whereas it is only applicable in case of accidental release of soybean A2704-12. Therefore, the Biosafety Advisory Council interprets this sentence with the following meaning : "In case of accidental release in the situation of no cultivation, ... the likelihood of unintended environmental effects of the soybean A2704-12 in Europe will probably be very low."

General conclusion

Based on the scientific assessment of the dossier done by the Belgian experts,
Taking into account the opinion of EFSA's GMO scientific panel, its answers to the questions raised by the Belgian experts, and considering the data presently available,
The Biosafety Advisory Council:

Agrees with the conclusion of the GMO panel of EFSA that: "it is unlikely that soybean A2704-12 will have any adverse effects on human and animal health or on the environment in the context of its proposed uses" provided that the applicant completes the compositional analysis of *toasted meal* with data of the herbicide treated transgenic soybeans in order to demonstrate the substantial equivalence .

In addition, the Biosafety Advisory Council is of the view that EFSA should advice future applicants
a) to complete the compositional analysis of the food with data on dietary fibre.
b) to present compositional data of non-transgenic, treated and non-treated transgenic lines in a more clear and transparent way in order to prevent confusing interpretations.

p.o.

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

Annexes :
- Minority declaration from a member of the Council
- Full comments of experts in charge of evaluating application EFSA/GMO/NL/2005/18 and comments submitted on the EFSAnet (ref: BAC_2006_PT_373)

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Minority Declaration of a member of the Belgian Biosafety Advisory Council on the application EFSA/GMO/2005/18 (A2704-12 soya)

In this soya file, no toxicological study was performed .

According to the guiding rules of the EFSA, toxicological studies are not to be required from the notifiers when they present compositional analyses not showing a significant difference between a GM product and its not GM counterpart (one speaks then about *substantial equivalence*) . According to the EFSA's opinion on this soya file , no significant change in composition was detected (what our Biosafety Council will possibly confirm in any event only at the condition of obtaining complementary data) .

But the guiding rules of the EFSA are not legally constraining documents . We are thus not obliged to approve this consideration of the EFSA on the absence of need for toxicological studies in the case of *substantial equivalence* .

It is scientifically reasonable to consider that the compositional analyses carried out are not precise enough to reveal the potential presence of a toxic substance which would be present in small concentration and effective as such . On the basis of the precautionary principle, one can thus consider that in vivo studies of potential toxicological impacts of the complete extracts likely to be found in the human or animal food and/or feed should always be carried out in GMO files intended for consumption (and this, by respecting protocols approved by professionals on the field) ; this should have been done in this precise soya file .

Bioveiligheidsraad
Conseil de Biosécurité

09 May 2006

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2006/PT/373
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Comments of experts in charge of evaluating the application EFSA/GMO/NL/2005/18
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 20 March 2006
Coordinator: Prof. Thierry Hance
Experts: Jean-Pierre Hernalsteens (VUB), Andre Huyghebaert (UGent), Jean-Marie Saint-Rémy (KUL)
Domains of expertise of experts involved: Ecology, population genetics, plant-insect relations, nature conservation, genetic engineering, human nutrition, biochemistry of food/feed, immunology, alimentary allergology, risk analysis, industrial processing, traceability of alimentary chain, soybean
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/NL/2005/18** concerns a notification of the company **Bayer CropScience** for the marketing of the genetically modified **soybean A2704-12** for food and feed applications under Regulation (EC) 1829/2003.
The notification has been officially acknowledged by EFSA on 10 February 2006.
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 notification on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. Its was expected that the expert should evaluate if the information provided in the notification 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 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 placed on the EFSAnet are indicated in grey. 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)

The dossier is complete and clearly written. It gives sufficient information and experimental results to allow a sound evaluation. The content is in accordance with the published information and my personal experience in the field. Based on the aspects that I evaluated (molecular characterisation and environmental safety), I have no objection against the importation and commercialisation of seeds of this soybean line for use as food and feed, as requested in the dossier.

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

Comments/Questions of the expert(s)

An overview of the use, properties, technology and applications of soybeans is given. The presence of anti-nutrients and their significance in nutrition is discussed in B.7.2.2 and annexes.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

1. The presence of a T-DNA border sequence in the vector used for the transformation by particle bombardment is surprising: it should only be useful for *Agrobacterium*-mediated transformation. As this has no influence on the safety of the transformation event or the resulting transgenic plants it should not be considered as problematic.
2. The vector used for transformation contains a beta-lactamase gene. This is inactivated by restriction enzyme digestion before the transformation and is present as two separate segments in the resulting transgenic plant. There is no known natural mechanism for rejoining these two fragments into an active gene, which could be transferred again to a bacterial host and expressed. Even if this would occur, this would not have significant implications for human or animal health, as beta-lactamase resistance is already widespread in bacterial populations, including pathogenic and commensal isolates from humans and domestic animals. Therefore in my opinion the presence of these beta-lactamase gene fragments has no influence on the safety of these transgenic plants. Nevertheless, taking into account the perception of the public about the presence of antibiotics resistance genes in transgenic plants, it would have been better to avoid this issue by the use of an other vector or fragment for transformation.
3. The 5' and 3' regulatory elements of the CaMV P35S promoter are used to control the expression of the herbicide resistance gene *bar*. There is in my opinion no valid scientific argument against the use of this promoter. However, it is possible that its 'viral' origin will be considered as problematic by part of the public.

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)

1. The dossier clearly arguments that the only trait in the transgenic plants that was modified is its herbicide resistance, which was achieved by the transfer and expression of a single herbicide modifying enzyme. There is, to the best of my knowledge, no reasonable doubt about the safety of this resistance mechanism, which is already applied on a large scale.
2. The insertion of the transgene took place in a nuclear DNA sequence homologous to chloroplast DNA. Insertion in such DNA, that has no known function in the nucleus, makes it very unlikely that the integration has caused a significant change in the properties of the transgenic plant or its seeds. This is in accordance with the results on the agronomic characterisation of the plant that are reported in the dossier.

D.2. Information on the sequences actually inserted or deleted

Comments/Questions of the expert(s)

The information available in the dossier seems to be complete and correct.

D.3. Information on the expression of the insert

Comments/Questions of the expert(s)

1. Stable transgene expression at a sufficient level is crucial for the engineering of herbicide resistant plants. This was tested and confirmed extensively during the development of this transgenic line.
2. Additional open reading frames are present in the modified region of the transgenic plant genome. I agree with the arguments given in the dossier, based on the analysis of the nucleotide sequence, that it is very unlikely that these will be expressed and lead to the production of a significant quantity of a polypeptide. Even if this would occur, it is again unlikely, by the nature of the sequences that are involved, that this polypeptide would have a biological activity that is relevant for the safety or intended use of the plant or its seeds.

D.4. Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability

Comments/Questions of the expert(s)

1. This is in principle not relevant for this dossier because only the use of the seeds and not the culture of the plants is intended.

2. In case seeds would be accidentally released into the environment, if no herbicide is applied, the expression of the *bar* gene will not influence the reproduction, dissemination or survival of the plant. The herbicide resistance is not problematic, as soybeans are not known to behave as weeds. If required, resistant soybeans can easily be controlled mechanically or by another herbicide.

D5. Genetic stability of the insert and phenotypic stability of the GM plant

Comments/Questions of the expert(s)

1. The transgene is integrated in the nuclear genome of the plant by an illegitimate recombination event. Such transgenes are typically stably integrated and behave as simple Mendelian genetic markers.
2. Stable expression of the transgene is easy to test by herbicide applications and has obviously been confirmed extensively in field tests before the line was accepted and released for agricultural use.
3. This is in principle not relevant for this dossier, because only the use of the seeds and not the culture of the plants is intended.

Additional comments from the coordinator

Soybean is a leguminous plant and it can fix atmospheric nitrogen as a source of nitrogen for growth and development in a symbiotic relationship with *Bradyrhizobium japonicum* as clearly indicated in the dossier. However no data are available on possible gene transfer to that bacteria during symbiosis or to another equivalent bacteria that could be present in other *Leguminosae* species.

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

Comments/Questions of the expert(s)

No such change caused by the expression of the transgene can be anticipated.

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)

No comments.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

No further comments.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Results are given of a comparative analysis of the non-transgenic and the transgenic, sprayed and non-sprayed, soybeans. As a comparison literature data are included.
The whole range of constituents is analysed including proximates and minor constituents.
In most cases there is no statistical difference between the analyzed samples. Results are also well in agreement with literature data. In case of a difference the result is discussed.
I used the document " Food Nutrition Evaluation"[1] as a guide.

Proximates:
No differences found. Fibre is expressed as acid detergent and neural detergent fibre. To my understanding this approach is particularly used for animal feed. In human food studies the concept of dietary fibre is widely accepted: food nutrition tables, food labeling …Very often a further specification as soluble and insoluble fibre, and even individual main constituents is applied.

Question: why are data given as acid and neutral detergent fibre whereas the dietary fibre is widely used in human food studies?

Minerals and vitamins:
No difference found for the constituents studied, with the exception of folic acid and vitamin E.
Most relevant minerals are included. However some important minerals are not given such as Mn, Cu, Zn and I.

Question: are data available for these minerals? If not why are they not considered as relevant?

Vitamins:
The analysis is limited to three water soluble and one fat soluble vitamin. No difference is found for vit B1 and B2. The level of folic acid in the non transgenic bean is rather low. For vit E the amount for the sprayed transgenic beans is low.

Question: Vit E or tocopherols are important constituents. In soybeans a range of tocopherols are present. How is de vit E capacity assessed? Are all tococpherols included or only α-tocopherol? Soybean contains significant levels of δ-tocopherol with a low vitamin but a high antioxidant capacity. Are data available for δ-tocopherol?

[1] In:The safety assessment of genetically modified crops for food and feed use, available at: http://www.biosafety.be/gmcropff/EN/NF/GuidanceNotes/GuidanceFF.htm

Amino acids:
No differences are found. Essential amino acids are covered.

Fatty acids:
No significant differences found. Important fatty acids are covered.

Anti-nutrients:
Relevant anti-nutrients have been studied. No significant differences have been identified. The lectin level of the considered soybean variety seems to be very low. Data of saponins are not included.

Question:
Are data about saponins available?

Isoflavones:
These important compounds are well studied. Levels found are generally within the range of standard values. There is a reasonable explanation when differences have been identified.

Additional comments from the coordinator

As this plant is tolerant to glufosinate ammonium, it may be expected that crops of the ACS-GMØØ5-3 variety shall be sprayed by this herbicide. No indication on potential presence and concentrations of glufosinate ammonium or other metabolites in the seeds following a regular cultivation process of such plants is however given. As that GMO was conceived to be used with that herbicide, such information is essential. If any residues are currently present in such seeds then toxicological implications should be given.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

No comment.

D.7.5 Product specification

Comments/Questions of the expert(s)

No comments.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

The PAT protein is detectable in whole soybeans and hulls and in defatted non-toasted meal and isolate. It is not detectable in a series of end products such as defatted toasted meal and other products.

This may be the result of protein denaturation or the physical separation process applied. This aspect is relevant for intake studies, among others.

Question:
Is there any further information why the PAT protein is not detectable in particular fractions? Non detection due to absence below detection level or due to presence in a denatured form?

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

No further comment unless the question under D.7.6.

D.7.8 Toxicology

Comments/Questions of the coordinator **(T. Hance)**

What about the presence of glufosinate ammonium residues or other metabolites in the seeds following a normal crop production process and their potential interaction with Pat proteins in toxicity?

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

There is sufficient information available in different crops and test systems to argument the safety of the protein encoded by the *bar* gene. This is, based on the molecular analysis, the only new protein that can be reasonably expected in these plants.

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)

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

This is a well presented dossier, which fulfills the criteria as classically defined for allergenicity testing. Such criteria are however of very limited value for the prediction of allergenicity. The expert realizes that there is no definition for allergens, as allergy results from an interaction between a protein and both the innate and adaptive immune systems, including genetic susceptibility and cytokine production. The text should include a short introduction stating that methods to predict allergenicity are of limited values. More precisely:

1. the search for sequence homology with allergens, or homology between epitopes binding IgE antibodies. Most IgE binding epitopes are conformational and discontinuous, which would escape detection by current homology search. T cell epitopes are not searched for; however, several algorithms are freely available and it is recommended to use them. The capacity to elicit a Th2 cell activation is indeed the driving force towards the formation of IgE antibodies.
2. glycosylation: many major allergens are not glycosylated
3. heat stability: most allergens are destroyed by heating, but there are notable exceptions, such as for peanut allergens. Again, this is a weak argument.
4. digestibility: not all proteins are digested by passage through the stomach and intestine. Some allergens such as lactalbumin can even be found in the milk of lactating mothers. As trace amounts of allergens are sufficient to elicit an allergic reaction (IgE-mediated), the digestibility assessment is of limited value.

Additional comments from the coordinator

What about the presence of glufosinate ammonium residues or other metabolites in the seeds following a normal crop production process and their potential interaction with Pat proteins in allergenicity?

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

In addition to the above mentioned results further analyses are related to raw products and products obtained after processing like raw soybeans, hulls, defatted toasted meal, oil after refining, bleaching and deodorization and soy isolate.
The nutritional value was further demonstrated in a poultry feeding study.

No comments in addition to my comments under D.7.3.

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)

The presence of the insert will give a strong selective advantage in presence of the relevant herbicides. Under other conditions, it will not affect the properties of the plant.

Additional comments from the coordinator

Is there any information on the potential interaction with the symbiont bacteria involved in nitrogen fixation?

In the dossier, it is claimed that as the insert gene is directed toward an herbicide and that in consequence no interaction are to be expected with insect or pathogens. No evidence is given to prove that claim. Several herbicides are known to have side effect on invertebrates or fungi.
Those comments apply in case of accidental dissemination of the GM plant.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

I agree with the information given in the dossier. The transgene is stably integrated in the nuclear genome and will only be transmitted by classical genetic crosses. No related wild plants, that could act as recipients for pollination, are present in the environment in the European Union. Therefore the only possible recipient of the transgene is cultivated soybean (*Glycine max*).

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 coordinator

In the dossier, it is claimed that as the insert gene is directed toward an herbicide and that in consequence no interaction are to be expected with insect or pathogens. No evidence is given to prove that claim. Several herbicides are known to have side effect on invertebrates or fungi.
Those comments apply in case of accidental dissemination of the GM plant.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

No comment.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

No comment.

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)

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)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

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

none