Bioveiligheidsraad
Conseil de Biosécurité

08-09-2015

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2015_0597

Title: Draft Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/BE/2011/98 from Bayer CropScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/BE/2011/98 was submitted by Bayer CropScience on 24 June 2011 within the framework of Regulation (EC) No. 1829/2003[1] for authorisation for import, processing, and food and feed uses (excluding cultivation in the EU) of herbicide-tolerant genetically modified (GM) soybean FG72.

Soybean FG72 was developed by biolistic transformation to express the HPPD W336 and 2mEPSPS proteins, which confer tolerance to isoxaflutole- and glyphosate-based herbicides.

The application was officially acknowledged by EFSA on 24 October 2011. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of GM organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). 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 13 January 2012.

The opinion of the EFSA GMO Panel was adopted on 25 June 2015 and published on 16 July 2015 (EFSA Journal 2015; 13(7):4167[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

On 22 July 2015 the EFSA opinion and the responses from the EFSA GMO Panel were forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

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

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1)
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/4167.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

Scientific evaluation

1. Environmental risk assessment

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

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The Biosafety Advisory Council is of the opinion that the compositional analysis of GM soybean FG72 does not raise safety concerns.

3.2. Assessment of toxicity

Soybean FG72 was developed to express the HPPD W336 and 2mEPSPS proteins. Based on previous positive assessments of the protein 2mEPSPS and taking into account the information provided by the applicant on the HPPD W366 protein, the Biosafety Advisory Council is of the opinion that in the context of its intended uses GM soybean FG72 does not raise safety concerns regarding toxicity.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that GM soybean FG72 would have an allergenic profile that would be significantly altered in comparison with its conventional counterpart.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that there are no indications that the GM soybean FG72 would be less nutritious than 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 EFSA opinion, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the questions of the EFSA GMO Panel and considering the

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

data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM soybean FG72 is unlikely to pose any risk to human and animal health.

Given the scope of the application of this GM soybean (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 soybean FG72 is unlikely to pose any threat to the European environment.

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex 1 : Minority declaration
Annex 2 : Compilation of comments of experts in charge of evaluating application EFSA/GMO/BE/2011/98 and comments submitted on the EFSAnet (ref. WIV-ISP/41/BAC_2012_0043)

Minority declaration of Philippe Baret

The compositional comparison of GM soybean FG72 and control plants is significantly different for several endpoints. In consequence, it is impossible to demonstrate substantial equivalence and a full toxicological analysis should have been performed. As such analysis is not provided by the notifier, a toxicological risk cannot be fully excluded. In consequence, my advice is negative on the authorisation of GM soybean FG72.

Bioveiligheidsraad
Conseil de Biosécurité

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2012_0043
Email. : bac@wiv-isp.be

13-01-2012

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2011/98 and Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 16 November 2011
Coordinator: Dr. René Custers
Experts: Armand Christophe (UGent), Jacques Dommes (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, breeding techniques, plant biology, human nutrition, animal Nutrition, biochemistry of food/feed, analysis of food/feed, industrial processing, Toxicology in vivo & in vitro, Immunology, alimentary allergology , plant allergens
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/BE/2011/98** concerns an application of the company **Bayer CropScience** for the marketing authorisation of the genetically modified **Soybean FG72** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 24 October 2011.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its

intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

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

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

There is little chance that the use of genetically modified soybean FG72 will be detrimental for animal and human health, based on a series of studies, including:

- Acute oral mouse and rat allergenicity and toxicity studies
- Biochemical and molecular characterization
- Search for homology to known toxins and allergens that affect animal or human health
- A lack of stability during processing

Furthermore, the safety of 2mEPSPS protein has already been assessed as safe (EFSA, 2009).

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No comment (information is adequate)

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

Comments/Questions of the expert(s)

Comment 1

No comment (information is adequate)

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Page 57, line 34. Printing error: proce**e**ded.

Comment 2

No comment (information is adequate)

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

No comment (information is adequate)

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

Comments/Questions of the expert(s)

Comment 1

Applicant used adequate methods (hybrization on Southern blots, PCR and DNA sequencing) to show the organisation of the transferred DNA, the translocation event that occurred during transgene insertion and the absence of vector backbone in the transgenic plant. Junction regions between insert and plant genomic DNA, as well as junctions between translocated DNA and genomic DNA were PCR amplified and sequenced. Adequate bioinformatic tools were used to prove that no wild type gene was interrupted, that not any new potential gene was created and that none of the potential ORF showed significant similarity with known toxins and allergens.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Matrix	Growth stage	2mEPSPS protein content			
		µg/g fresh weight		µg/g dry weight	
		Average ± SD	Range	Average ± SD	Range
Leaf	V4	90.4 ± 26.1	44.9 – 152	569 ± 164	283 – 958
	V6	79.1 ± 29.6	39.2 – 136	437 ± 163	216 – 753
	V8	115 ± 38.2	60.5 – 203	668 ± 222	351 – 1180
Stem	V4	18.8 ± 6.16	6.08 – 31.3	211 ± 68.9	68.0 – 350
	V8	13.4 ± 2.62	8.71 – 17.3	117 ± 22.9	76.1 – 151
Root	V4	4.89 ± 1.99	1.63 – 8.21	32.5 ± 13.2	10.8 – 54.5
	V8	5.75 ± 2.31	2.62 – 10.7	43.7 ± 17.6	19.9 – 81.2
Seed	NA	2.37 ± 0.75	1.34 – 3.74	2.62 ± 0.83	1.48 – 4.13

Table 17. Amounts of 2mEPSPS and HPPD W336 in FG72 Soybean Grain (Martone, 2011 M-405348-01-1).

Protein	Treatment		Fresh Weight (ng/g)	Dry Weight (ng/g)	% Crude Protein
2mEPSPS	Unsprayed	Range	70,400 – 151,000	79,500 – 171,000	0.0171 – 0.0384
		Mean ± SD	118,000 ± 18,600	132,000 ± 20,800	0.0306 ± 0.0054
2mEPSPS	Sprayed	Range	91,400 – 160,000	101,000– 176,00	0.0229 – 0.0427
		Mean ± SD	128,000 ± 21,400	142,000 ± 23,500	0.0330 ± 0.0063
HPPD W336	Unsprayed	Range	761 – 1550	844 – 1720	0.00024 – 0.00041
		Mean ± SD	1220 ± 205	1370 ± 231	0.00032 ± 0.000050
HPPD W336	Sprayed	Range	1170 – 2060	1290 – 2310	0.00030 – 0.00051
		Mean ± SD	1590 ± 253	1770 ± 288	0.00041 ± 0.000058

The first table (table 8 in T.D.) mentions an average amount of 2mEPSPS in seed of 2.62 µg/g d.w., whereas the second table (table 17 in T.D.) indicates an average amount of 140000 ng/g d.w. which equals 140 µg/g d.w.. **Is there an explanation for these different data?**

Comment 2

Note that the level and range of 2mEPSPS is quite different in grain when soy FG72 is grown under greenhouse conditions (Part I, Table 8, page 63) than under “field conditions” (Part I, Tables 9 and 10, page 64-65). Thus differences in “environmental field conditions” might have a larger effect than noted (Part I, Tables 9 and 10, page 64-65) on the level of this protein.

Comment 3

No comment (information is adequate)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

Comment 1

Stabilities of the insert and of the phenotype were adequately proved.

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

Comments/Questions of the expert(s)

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

Although some statistical significant differences occurred, the mean values are always within the range provided by the commercial products and ranges in the published literatures.

I do have a question concerning the amount of trypsin inhibitor. In un-toasted meal this amount is a factor of about 3 lower in FG72 compared to its non-GM counterpart. In toasted meal this kind of difference is not observed. Can this difference be explained?

Comment 2

A traditional and logical approach is followed in the comparative assessment. Key nutrients and other significant components of soybean FG72 grain were compared with grain from the non-GM conventional counterpart Jack.
Grain from three commercial varieties, cultivated under the same conditions, were also included. If available, data from literature were also considered.

Comment 3

The choice of the non-GM counterpart (Jack) is logical. OECD 2001 guidelines were followed for the selection of components to be analysed. Yet, since these guidelines were issued, soy saponins have been demonstrated to be an anti-nutritional factor for fish (Knudsen et al., 2007; Wei et al., 2011). Therefore it seems warranted that determination of saponins in soy would become mandatory in the future.

Additional comment from SBB:
For consistency with previous dossiers we suggest to transmit the following comment:
"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

Field trials include ten locations in 2008 and six locations in 2009 in a randomized block design. Soybeans were harvested from the non-GM conventional counterpart treated with conventional herbicides, the soybean FG72 treated with conventional herbicides and the soybean FG72 treated with the intended herbicides (IFT and GLY). Three non-transgenic commercial soybean varieties were planted at the same locations but were not included in the block design.
The statistical analysis applied is fully explained.
I have no further questions on this item.

Comment 2

No questions

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

The OECD consensus document from 2001 is followed. Components analyzed include:

- proximate and fibre compounds,
- micronutrients: minerals and vitamins,
- isoflavones,
- anti-nutrients
- amino acids,
- fatty acids.

Compounds analyzed in raw soybeans, in processed soybeans and forage, are summarized in a table. Commodities analyzed include, in addition to raw soybeans, hulls, meal, toasted meal, protein isolate, crude oil, refined oil, crude lecithin and forage.

The selection of compounds for the specific commodities is logical.
I have no further comment on this item.

Results

Proximates include moisture, crude protein, total fat, ash, acid detergent fibre and neutral detergent fibre.

Fibre constituents are assessed by methods not accepted anymore in human nutrition.
This is in contrast with previous dossiers in which fibre constituents were determined according to up to date methods.
A similar observation applies for carbohydrates. The indirect method by difference is not applied anymore in terms of human nutrition.

Additional comment from SBB:
The fibres have been analysed according to the recommendations of OECD.

In most cases no significant differences were found between the sets of transgenic and non transgenic groups. If a significant difference is observed as for ash, the values are within the range of commercial products and literature data.

Amino acids are an important item for protein sources like soybeans. All essential and "semi-essential" amino acids are included.
In most cases no significant differences were found between the two transgenic and the non-transgenic control soybean.
The applicant formulates a reasonable explanation for the cases were significant differences were observed.
No further comment.

Fatty acids are also very important constituents in soybeans. Relevant saturated, mono-unsaturated and poly-unsaturated fatty acids were analyzed.
I agree with the conclusions of the applicant.

Vitamins measured are vit A, B1, B2, K, folic acid and the range of tocopherols: total, alpha, beta, gamma and delta.
I have no comment on the selection of vitamins and the conclusions of the applicant.

Minerals analyzed are calcium, phosphorous, potassium, magnesium, sodium and iron.
The applicant concludes in the same direction as in previous cases.

One can always question if all relevant minerals and vitamins are included. Some other constituents could be considered.
In my opinion the selection of compounds is logical.

Isoflavones are very well kown constituents of soybeans. Daidzein, genistein and glycitein were analyzed. They are present as glucosides and as esters. Results are expressed as aglycon equivalents. This approach is valuable.

In most cases no significant differences were found. In some cases differences found were significant. The applicant states that the differences are within the reference ranges and are not considered to be of biological importance.
I agree with this conclusion.

Endogenous toxins and antinutrients levels were also studied. Analysis include phytic acid, raffinose, stachyose, trypsin inhibitors and lectin.
As in previous cases no significant differences were found. If it is the case the values are within the range of literature data.
To my knowledge raffinose and stachyose are the most relevant flatulence factors in soybean. Any information on verbascose, the third but minor flatulence factor in soybeans, is missing in the dossier. However data on verbascose would have no influence on the conclusion.

Soybeans products and forage
A similar but adapted approach was followed for soybean products and forage. The applicant concludes in the same direction: no major differences between the transgenic and non-transgenic samples.
This chapter contains information on the major phosphatides in crude lecithin, an important by-products in soya processing. As far as I remember data on phosphatides were not included in previous similar dossiers. This is one of the first dossier with information about this important compound.

Conclusion
The applicant concludes that, based upon the analytical data, the statistical analysis and an assessment of the nutritional impact of the observations, soybean FG72 is found to be nutritionally equivalent to the non-GM conventional counterpart.

I have some (minor) comments on the methodology used in the assessment of carbohydrates, fibre constituents and antinutrients.

I basically agree with the conclusion of the applicant.

Comment 2

1)Table 28, page 91:
a) note that all saturated fatty acids are significantly increased at the expense of palmitic acid. This points to increased elongation activity (Schreiber et al., 2005). The differences noted in fatty acid composition pose no health problems. Yet the question remains whether a plausible explanation can be given for this unexpected finding.
b) two different isomers of C18:1 are present in soybean oil (oleic acid and cis-vaccenic acid; the latter fatty acid about 5% of the former) (Ezeagu et al., 1998). If the higher value of C18:1 found in seed of FG72 soybean compared to Jack (about 24% vs 22%) would be due to an important increase of cis-vaccenic acid, the claim that FG72 soy is substantially equivalent with its non-transformed counterpart could no longer be maintained. Although the presence of cis-vaccenic acid in food poses no health problem, at least a plausible explanation would be required in case this fatty acid would be increased. Because no difference between both isomers was made in the application (which by the way can be easily determined), this possibility can not be excluded.
c) note that there are small differences in Table 8 and in the original study refered to (Oberdörfer 2010 in references to Part I) (e.g. range for sum saturated fatty acid = 9.43-23.55 in Table 8 vs 9.95-23.03)

2) Table 46, page 107: Note that the concentration of L-alpha-phosphatidylethanolamine in Jack is outside the range (lower) given by Oberdoerfer which is claimed to be a literature range. As Jack soybean is a non-transformed soybean variety, this raises the question which values were used to determine the "literature range".

3) Editorial comment: page 90, 2nd line from the bottom: serine should be replaced by lignoceric acid.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comments

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Relevant aspects are covered in 7.3.

Comment 2

No questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Table 52. Calculation of the maximum theoretical 2mEPSPS and HPPD W336 protein amounts in animal diets produced with FG72 seeds

	2mEPSPS in µg/g Animal Diet				Percentage of 2mEPSPS in the Animal Diet			
	Beef Cattle	Dairy Cattle	Poultry	Swine	Beef Cattle	Dairy Cattle	Poultry	Swine
Seeds	36	36	48	60	3.6×10^{-3}	3.6×10^{-3}	4.8×10^{-3}	6.0×10^{-3}
	HPPD W336 in µg/g Animal Diet				Percentage of HPPD W336 in the Animal Diet			
	Beef Cattle	Dairy Cattle	Poultry	Swine	Beef Cattle	Dairy Cattle	Poultry	Swine
Seeds	0.20	0.20	0.26	0.33	2.0×10^{-5}	2.0×10^{-5}	2.6×10^{-5}	3.3×10^{-5}

Is it possible to make an estimation of the effective average intake of both proteins (expressed as µg per day) for the different types of animals?

Comment 2

Comment concerning footnote “b” to Table 5 (Part I, page 112). In contrast to the statement of the applicant, whole soybeans are consumed in Europe and intake data have been published (Kleinan-Boker et al., 2002). The actual intake in some European populations may be 6 times higher (e.g. 0.66 g/ person/day in The Netherlands) than the “default” value used by the applicant (0.1g/person/day).

Comment 3

No comments.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Data gap: Information is lacking on the levels and fate of herbicide residues (glyphosate, isoxaflutole, diketonitrile) in crop tissues.
Although the effect of herbicides on human and animal health falls under Directive 91/414/EC, it is the duty and responsibility of the toxicologist assessing the risk of the genetic modification to evaluate and discuss the complete picture of the genetic modification.
Rationale: The GM soybean plant is developed to be able to use the herbicides glyphosate and isoxaflutole. Data concerning the use of the herbicides in the field trials is available. However, no data is made available concerning the identification and quantification of the herbicides and metabolites residues in the GM plants and grain used for food/feed. As the use of these herbicides is linked to the genetic modification, the applicant should make the residue data available and make an estimation of the anticipated intake (food/feed).

Additional comment from the coordinator:
The assessment of the safety of the herbicide and its residues is outside the remit of the Biosafety Council.

Comment 2

The HPPD W336 protein was produced in E. coli. Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins. However, there has been an extended assessment of the toxicity of HPPD W336, based on a series of studies:
1) characterization of the biochemical properties
2) absence of biologically relevant amino acid sequence homology with known toxins and allergens
3) in vitro heat stability study
4) in vitro digestibility in simulated gastric and intestinal fluids
5) absence of indications of toxicity in an acute oral toxicity study in mice
6) absence of indications of toxicity in a repeated dose 28-day study in mice
7) the activity dropped below 50% after the protein was incubated at 45°C for 20 minutes. At more elevated temperatures (60°C and 95°C), the HPPD W336 activity was abolished after 2.5 minutes
8) the protein was only active within a narrow pH interval of 5 to 8.5.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Newly expressed proteins: HPPD W336 protein, 2mEPSPS protein
HPPD W336 protein
The safety of the HPPD W336 protein was demonstrated by biochemical characterisation, by an amino acid sequence homology search with known toxins and allergens, an *in vitro* heat stability test and *in vitro* digestibility testing, acute and subacute toxicity testing in mice.
Comment: The by gavage administration of the limit dose of 2000 mg HPPD W336 protein/kg bw in female OF1 mice (n=5) in the acute toxicity study (Rascle, 2009) resulted in decreased spleen weight (absolute and relative) although without gross or histopathological related findings. In contrast, in the 28-day oral toxicity study (Kennel, 2010) the administration of HPPD W336 protein given by gavage (limit dose, 1000 mg/kg bw/d) to male and female mice (5/sex/group) did not result in an effect on the spleen (absolute and relative spleen weight, histopathology). Nevertheless, a decrease in aspartate (22%) and in alanine aminotransferase (31%) was observed in male mice although without related histopathological findings and only in this sex.

2mEPSPS protein
The assessment is adequate and acceptable. No further comments/questions.

Comment 2

The amino acid sequence of the 2mEPSPS protein expressed in FG72 soybean is identical to the 2mEPSPS protein expressed in GA21 maize and GHB614 cotton.

a) Degradation of the HPPD protein in simulated gastric fluid (Rascle, 2009 (M-356196-01-1)).

HPPD W336 protein was very rapidly digested in.

b) Degradation of the HPPD protein in simulated intestinal fluid (Rascle, 2009 (M-356198-01-1)).

This study indicated a complete digestion of the HPPD W336 protein within less than 30 seconds in presence of pancreatin.

c) HPPD: Acute Oral Toxicity Study in Mice (Rascle, 2009 (M-358598-01-1)).

Conclusion:
There were no adverse effects of the protein when administered by oral gavage at a dose of 2000 mg/kg female mice.

d) HPPD: Repeated dose oral toxicity (28-day feeding) study in mice (Kennel, 2010 (M-368158-01-1)).

Conclusion: Although some differences between control and test group occurred, these seem to be unrelated to the introduction of the genetic modification.

e) HPPD: Assessment of Amino Acid Sequence Homology with Known Toxins (Rascle, 2011 M-355651-03-1).

From the TECHNICAL DOSSIER: "The HPPD protein showed important similarities with proteins in *Vibrio vulnificus*, a pathogenic bacterium present in seawaters and able to infect humans who consume seafood (Chang *et al*., 1997) and legionella.
These observations support the hypothesis that VLLY and LLY proteins are HPPDs and therefore, share typical HPPD structure with HPPD W336. This is corroborated by several authors who demonstrated the HPPD activity of the legiolysin (Wintermeyer *et al*., 1994; Steiner *et al*., 2001). In addition, although VLLY or LLY protein expression was shown to be necessary for the hemolytic activity of bacteria, the direct hemolytic activity of these proteins was not observed (Wintermeyer *et al.*, 1994; Chang *et al.*, 1997; Steinert *et al.*, 2001). It was shown that homogentisic acid, the product of HPPD enzymatic activity, forms spontaneously plasma soluble, toxic melanins that have hemolytic activity (Hegedus and Nayak, 1994). This supports the hypothesis that HPPD is not directly hemolytic."

The herbicide tolerant HPPD is introduced into soybean, which makes it able for the plant to continue producing homogentisic acid. **Is production of toxic melanins not possible in soybean ?**

Additional comment from the coordinator:
Homogentisic acid is the product of HPPD in both non-transgenic and transgenic plants. So I don't see the relevance of this question for the safety assessment of the FG72 soybean.

Comment 3

In my opinion, all results presented are in line with a safe hazard profile of the newly expressed proteins.
Printing error. Part I, page 119, line 7:CO_2

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comments/questions.

Comment 2

No new constituents have been detected in FG72 soy products nor are they expected. This is not an absolute prove that they are not formed. However, the poultry study and rat study indicate that soy FG72 is safe.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No particular natural constituents of FG72 soybean are considered to be of significant concern to require additional information or further risk assessment. No further comments/questions.

Comment 2

See D.7.3 comment 1.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Repeated-dose 90-day oral toxicity study in the rat (Odin, 2010)
The assessment is adequate and acceptable.
42-day poultry feeding study (Stafford, 2009)
The assessment is adequate and acceptable.
In conclusion: no potential health and food safety concerns have been identified.

Further comment: No data is made available concerning the herbicides glyphosate and isoxaflutole and their metabolites residues in the FG72 soybean grain used for food/feed.

Additional comment from the coordinator:
The assessment of the safety of the herbicide and its residues is outside the remit of the Biosafety Council.

Comment 2

a) 42-day feeding study in broiler chickens (Stafford, 2009 (M-358025-01-1)).
Conclusion: The growth and health of chickens on a diet containing FG72 toasted soybean seedmeal were comparable to chickens on two control diets, including a commercial variety of toasted soybean seedmeal and a non-transgenic, non-GM counterpart to the FG72 toasted soybean seedmeal.

b) 90-Day rat feeding study (Odin, 2010 (M-368148-01-1)).
Conclusion: No diet-related health effects were seen during this 90-day rat feeding study.

Comment 3

No questions

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

HPPD W336 protein was rapidly degraded in the SGF and SIF. However, this is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005). Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. 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.

Rouquié et al. (2010) found no significant increase in the level of allergens in FG72 soybean seeds, so that FG72 soybean can be considered as safe as its non-GM counterpart with regards to endogenous allergenicity. Nevertheless, they also mentioned the need for additional research to evaluate the biological variability in the levels of endogenous soybean allergens and the correlation between level of allergens and allergenic potential in order to improve the interpretation of the safety assessment of GM soybean.

Comment 2

The allergenic profile of FG72 was compared to that of its non-transgenic comparator. No significant differences were noted.
Part I, page 125, line 32: printing errors: l***o***w probabilities; line 33: replace I by *if.*

Comment 3

The genetic modification implemented in the FG72 soybean GM encompasses a supplementation of the plant with herbicide-resistant mutant enzymes that take over upon herbicide spraying the function of endogenous herbicide-sensitive enzymes. The wild-type exogenous enzymes clearly have a history of safety both regarding toxicology and allergenicity. Therefore, the mutations inserted and their possible consequences constitute the real concern regarding safety rather than the wild-type enzymes per sé.

For both the 2mEPSPS and the HPPD W336 proteins, the extensive *in silico* and wet bench analyses performed do not indicate the mutant proteins possess allergenic properties. Also testing of the whole GM plant for increased reactivity to sera from soybean allergic and sensitized individuals does not indicate increased levels in the GM of allergens already expressed in the parent soybean plants.

One concern however remains, namely to what extend did the inserted mutations affect the enzymatic activity and/or substrate specificity of the mutant enzymes. A modified enzymatic activity may increase the levels of rare intermediates and hereby generate new allergens. This issue was properly addressed for 2mEPSPS but not for HPPD W336. In the characterisation of the biochemical properties, the enzymatic activity of 2mEPSPS was compared to that of wild-type EPSPS, revealing no pronounced differences. However, such comparison was not made for HPPD W336. Pending such a biochemical comparison, it is impossible to fully exclude an increased risk for allergenicity of the whole plant due to a modified enzymatic activity of the mutant HPPD W336 protein.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

Some values showed significant differences for α-tocopherol (vitamin E) between non-GM conventional counterpart and FG72 soybean. Vitamin E can be seen as a compound with a nutritive value. This is in accordance with results of Matringe et al. (2005) for transgenic HPPD-PDH plants.

Table 32 of the dossier shows a lower calcium and magnesium content and a tendency for a lower sodium content: this is a consequence of the lower ash content: see P.84 of the dossier. The fact that calcium content is decreased, while the phosphorus content is not modified, merits some emphasis, because the calcium/ phosphorus ratio is important in animal nutrition. Nevertheless, the mean values are inside the range for commercial products.

Comment 2

No questions

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)

NOT APPLICABLE

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

D.9.1. Persistence and invasiveness

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

Based on the available data, no adverse effects on human health are expected.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

A dietary incorporation levels of approximately 20% for broilers was used for FG72 soybean meal in this dossier (Stafford, 2009), compared with dietary levels of 15-31% in an experiment of Sterling et al. (2002), as all supplementary protein came from soybean meal. However, I missed the feed ingredient composition of each treatment group for each sub-period.

No diet-related changes were observed in rats and broiler chickens fed event FG72 soybean meal incorporated at up to 15 or ±20%, respectively.

Comment 2

Because the levels of saponins in FG72 soy products were not measured, one can not be absolutely sure that their use will not have an adverse effect on fish fed in aquaculture (see D7.1).

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

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

EFSA, 2009. Application (Reference EFSA-GMO-NL-2008-51) for the placing on the market of glyphosate tolerant genetically modified cotton GHB614, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. Scientific Opinion of the Panel on Genetically Modified Organisms. EFSA Journal 985, 1-24.

Ezeagu IE, Petzke, KJ, Lange E, Metges CC, (1998). Fat Content and Fatty Acid Composition of Oils Extracted from Selected Wild-Gathered Tropical Plant Seeds from Nigeria. *JAOCS 75*, 1031–1035

Freese, W., Schubert, D. (2004). Safety testing and regulation of genetically engineered foods. In Harding, S.E. (Ed.) Biotechnology and Genetic Engineering Reviews 21: 299-324.

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

Keinan-Boker L, Peeters , Mulligan AA et al., (2002). Soy product consumption in 10 European countries: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 5(6B):1217-1226.

Matringe, M., Sailland, A., Pelissier, B., Rolland, A., Zink, O. (2005). p-Hydroxyphenylpyruvate dioxygenase inhibitor-resistant plants. Pest Manag. Sci. 61:269-276.

Meredith, C. (2005). Allergenic potential of novel foods. Proc. Nutr. Soc. 64: 487-490.

Rouquié, D., Capt, A., Eby, W.H., Sekar, V., Hérouet-Guicheney, C. (2010). Investigation of endogenous soybean food allergens by using a 2-dimensional gel electrophoresis approach. Regul. Toxicol. Pharmacol. 58: S47–S53.

Schreiber L, Franke R, Lessire R, (2005). Biochemical characterization of elongase activity in corn (Zea mays L.) roots. Phytochem 66**:** 131-138.

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.

Sterling, K.G., Costa, E.F., Henry, M.H., Pesti, G.M., Bakalli, R.I. (2002). Responses of broiler chickens to cottonseed- and soybean meal-based diets at several protein levels. Poult. Sci. 81: 217-226.