Bioveiligheidsraad
Conseil de Biosécurité

16-06-2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2011_0553

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2008/52 from Bayer CropScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2008/52 was submitted by Bayer CropScience on 3 April 2008 for the marketing of genetically modified soybean A5547-127 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean A5547-127 expresses the *pat* gene leading to the production of the enzyme phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate. The PAT enzyme confers tolerance to glufosinate-ammonium containing herbicides.

The application was officially acknowledged by EFSA on 18 July 2008. 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). Eight 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 28 October 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 10 May 2011 (EFSA Journal, 2011;9(5):2147)[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 May 11th, 2011 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. In addition, the complementary information regarding (i) molecular characterization and (ii) allergenicity testing sent by the applicant to EFSA in the course of the evaluation of the application was provided to the coordinator and to the experts who evaluated these aspects of the application. The comments 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.

[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/2147.htm>

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Scientific evaluation

1. Environmental risk assessment

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

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

The BAC agrees with EFSA that the presentation of data could have been improved. This in itself, however, does not raise any safety concerns.

3.1. Assessment of compositional analysis
The compositional analysis showed a number of statistically significant differences between the GM soybean A5547-127 and its conventional counterpart A5547. These differences were not consistent across sites and years and fell within the range of natural variation within soybean. The Biosafety Advisory Council is therefore of the opinion that these differences do not raise safety concerns.

The Biosafety Advisory Council also notes that the level of antinutrients in toasted meal of non-GM soybean that is reported in the application dossier, is unexpected. This, however, does not raise any safety concern about the GM soybean.

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[4], 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 documents 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. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

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

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

4. Monitoring

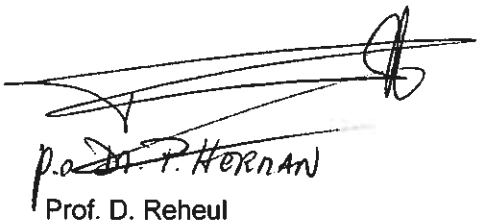
With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

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,

Agrees with the GMO panel of EFSA that

"genetically modified soybean A5547-127 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses".



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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2008/52 and *comments submitted on the EFSAnet (ref. BAC_2008_827)*

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Conseil de Biosécurité

28-10-2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2008_827
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Compilation of comments of experts in charge of evaluating the application EFSA/ EFSA/GMO/NL/2008/52
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 18 August 2008
Coordinator: Prof. Thierry Hance
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Johan Claes (KH Kempen), Jean-Pierre Hernalsteens (VUB), André Huyghebaert (UGent), Peter Smet (Consultant), Nancy Terryn (UGent), Michel Van Koninckxloo (HEPHO)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, human nutrition, biochemistry of food/feed, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, agronomy, crop protection management, agro-ecology, herbicide tolerance, soybean
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/NL/2008/52** concerns an application of the company **Bayer CropScience** for the renewal of the marketing authorisation of the genetically modified **soybean A5547-127** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 18 July 2008.
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

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

General information is clear and adequate. No questions.

Comment 2

In Section A.7, the conditions for placing on the market are discussed. Reference is made to the crushing facilities that are situated in areas where there is no agricultural activity. It seems to suggest that there is only a low risk for environmental impact. This is, however, not a valuable argument, since it can not be prohibited that soybean is transported elsewhere.

Comment 3

The information provided is sufficient.

Comment 4

The dossier is very clear and very well documented. The genetic modification for herbicide tolerance has been applied already in many similar events.

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

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

Comment 2

The information that is given in the dossier corresponds completely to the well-known biological and agronomic properties of soybeans.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

Comment 2

I agree with the dossier: the only trait that can be modified by the genetic modification is herbicide resistance, resulting from the expression of the PAT enzyme.
The presence of the right T-DNA border repeat sequence (RB) in the plasmid that was used for the transformation is not explained. To the best of my knowledge it is only involved in *Agrobacterium*-mediated T-DNA transfer (Wang *et al*., 1984, 1987) and has no function in the particle bombardment that was used in this study. The presence of this element has no consequence for biosafety.

Comment 3

The determination of inserted sequences with Southern blots in the soybean event of the application showed correctly the presence of one copy of the *pat* gene cassette and truncated parts of the *bla* gene at the 5' and 3' ends of the insert. The integration happened at a single locus that was confirmed by inheritance patterns. The insert was further characterized by determining the sequence of the inserted transgenic DNA in the event.

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

The only expected change of the phenotype of the transgenic plants is indeed resistance to phosphinothricin-related herbicides.

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

Comments/Questions of the expert(s)

Comment 1

The inserted DNA sequence was accurately studied by DNA hybridisation and by cloning followed by sequence analysis.

Although it has no implications for biosafety (see for instance Demanèche *et al*., 2008 and references therein), the presence of the two β-lactamase gene fragments is somewhat unfortunate. It would have been better to take the reluctance of consumers for any DNA related to antibiotics resistance into account and to avoid the presence of this sequence in the DNA sample that was used for the transformation.
According to the information that is provided it is very unlikely that any other protein than the PAT enzyme would be produced at a significant level in the transgenic plants.

Comment 2

In table 8 some bases are put in small letters some in large ones, should be mentioned in legend why that is.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

As expected for the constitutive P35S promoter, the PAT protein is properly expressed. It is unlikely that the expression of any of the open reading frames that are generated by the insertion of the insert would produce a protein with a biosafety-relevant biological activity.

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

As expected from the nature of he insert, the only difference between the transgenic line and its non-transgenic parental line is the herbicide resistance.

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

Comments/Questions of the expert(s)

Comment 1

The data that are provided are in agreement with the stable Mendelian transmission of the transgene.

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

Gene transfer from plants to bacteria is not excluded, but would occur at an extremely low frequency. This would in addition not lead to consequences that are relevant for biosafety because the transferred trait would not confer a selective advantage to the bacteria and would therefore most likely not be kept in the bacterial population.
Other soybean plants are the only possible partners for crossing. As the material will not be cultured in the EU and the structure and function of the flowers was not changed by the genetic modification (and gene transfer rates will therefore not be increased) this is very unlikely.

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

Comment 1

The dossier is well established and discusses the safety (toxicological, allergenicity, food/feed nutrition) with own experiments and based on literature. The issues indicated in the Guidance Notes of the Biosafety Council (The safety assessment of genetically modified crops for food and feed use, April 2003) are well discussed. Some minor comments are made regarding these topics.

For sections D.7.9 and D.7.10, there is insufficient information available in the technical dossier to allow an easy evaluation. The results can be searched for in the appendices, but a more detailed description of the results in the main dossier is advisable.

Additional comment from the coordinator

General comment to EFSA: Such dossier will be more easy to evaluate for experts if the recipient try to be more pedagogic and if the information needed is more easy to find. There is so many pages of appendices with various information that it is not easy to find the appropriate data we need..

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

It is suggested that saponins are included in the compositional analysis.
Indeed, saponins are present in soy in relatively high quantities (Berhow et al., 2006) and although poorly absorbed in humans (Hu et al., 2004) they can cause bloat in ruminants (Van Haver et al.,

2003) and induce enteritis in salmon (Knudsen et al., 2007). Soya sapogenols, obtained by hydrolysis of saponins, clearly have important biological effects (e.g. Zhang et al., 2008).

Remark SBB

For consistency with previous dossiers we suggest to transmit this comment 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 2

6a) Composition analysis of raw soybean seed.

Proximates		**Minerals**	
moisture	X	calcium	X
protein	X	copper	
fat	X	iron	X
ash	X	magnesium	X
carbohydrates	X	manganese	
acid detergent fiber (ADF)	X	phosphorus	X
neutral detergent fiber (NDF)	X	potassium	X
total detergent fiber (TDF)		selenium	
starch		sodium	X
		zinc	
		total nitrogen	
		chlorine	

Vitamins		**Amino acids**		**Fatty acids**		**Secondary metabolites**		**Antinutrients**	
A (β-carotene)		alanine	X	8:0 caprylic		ferulic acid	X	phytic acid	X
B1 (thiamine)	X	arginine	X	10:0 capric		furfural		raffinose	X
B2 (riboflavin)	X	asparagine		12:0 lauric		inositol	X	trypsin inhibitor	X
B3 (niacin)		aspartic acid	X	14:0 myristic	X	*p*-coumaric acid	X	gossypol	
B4 (choline)		cysteine	X	14:1 myristoleic				malvalic acid	
B5 (pantothenic a)		glutamic acid	X	15:0 pentadecanoic				sterculic acid	
B6 (pyridoxine)		glycine	X	15:1 pentadecenoic				dihydrosterculic acid	
B9 (folic acid)	X	histidine	X	16:0 palmitic	X			lectin	X
C (ascorbic acid)		isoleucine	X	16:1 palmitoleic	X			stachyose	X
E (α-tocopherol)	X	leucine	X	17:0 margaric	X				
Cryptoxanthin		lysine	X	17:1 heptadecenoic					
		methionine	X	18:0 stearic	X				
		phenylalanine	X	18:1 oleic	X				
		proline	X	18:2 linoleic	X				
		serine	X	18:3 linolenic	X				
		threonine	X	20:0 arachidic	X				
		tryptophan	X	20:1 gadoleic	X				
		tyrosine	X	20:2 eicosadienoic					
		valine	X	20:3 eicosatrienoic					
				20:4 arachidonic					
				20:5 eicosapentaenoic					

Vitamins		Amino acids		Fatty acids		Secondary metabolites		Antinutrients	
				22:0 behenic	X				
				22:1 erucic	X				
				22:5 docosapentaenoic					
				22:6 docosahexaenoic					
				24:0 lignoceric	X				

It can be concluded that the determined differences between A5547-127 and A5547 soybean seeds have no nutritional or biological relevance for humans or animals and are inside the range reported for commercial soybean varieties.

Comment 3

Soybean A5547-127 will be referred further as submitted soybean.
The submitted soybean was compared with the parent soybean A5547, referred as parent soybean.

Comment 4

The information provided is sufficient.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

The choice of the comparator is logical and description of production of material for comparative assessment is adequate.

Comment 2

Both soybeans were grown at different (16) locations during different (4) seasons.
At each location there were
- three plots of parent soybean
- three plots of submitted soybean
- three plots of submitted soybean, sprayed with glufosinate.

In the comparative analysis the submitted soybeans were compared with the parent soybeans. Data from literature were also used as a source of information about the composition.
No further comment

Comment 3

The information provided is sufficient.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

1. Including saponins in the compositional analysis is suggested (see above)
2. Using 3 data for descriptive statistics and assuming that they are normally distributed (Technical dossier, part I, page 65) and ANOVA of these data and using t-test for the significance of the means for site by site analysis is somewhat tricky. For instance, for the Arkansas site 403, the Total Vitamin E content of the 3 transgenic sprayed samples is 108 IU/kg DM; 91.6 and 59.3. (Bayer CropScience report 07 B 005 page 98). Can a normal distributing be assumed? Hopefully, this problem will be addressed in the new guidelines for statistical evaluation.
3. Evaluation of the results of Vitamin E values (Part I, Page 75, Table 25) leads to an interesting general question where a position has to be taken. What if a value of the non-treated GM falls in the published reference range but of the treated GM falls outside this range (the finding that the control and treated values are not significantly different due to the large standard deviations seems not to be very helpful in this respect). In other words: must substantial equivalence be shown between the comparator and commercial varieties on the one hand and the GM counterpart as it is intended to be grown on the other hand or grown without the intended treatment?

Comment 2

Table 22 (and the corresponding appendices of Rattemeyer-Matschurat (2008a)) show considerable differences between treated and non-treated transgenic soybean. The treated transgenic soybean is for oleic acid statistically different from non-transgenic soybean on more sites as compared to the non-treated transgenic soybean.
Is there any knowledge about the relation between the pat gene and the production of oleic acid? Is there any explanation for the difference between treated and non-treated transgenic soybean?

More information about this difference can be relevant, since it might reveal unintended biochemical pathways. It would make the dossier more convincing on this point if a possible biochemical pathway is discussed/hypothesized, based on a literature survey and/or experiments.

As is argued by the applicants, from a nutritional point of view, these differences are not relevant.

Comment 3

The OECD document was followed in the selection of constituents for analysis.

This analysis includes

proximates: moisture, protein, fat, ash, carbohydrates by difference, ADF, NDF

- no information about dietary fibre, as in previous dossiers

minerals: Ca, Na , K, Mg, Fe, P

- relevant minerals are included

vitamins: B1, B2, folic acid, vitamin E and tocopherols

- relevant vitamins are included as well as the tocopherol composition

antinutrients: stachyose, raffinose, phytic acid, trypsin inhibitor, lectins

- a broad range of anti-nutrients is studied,
- I wonder why the third flatulence factor verbascose was not determined

amino acid composition

- data cover the whole range of amino acids

fatty acid composition

- the range of fatty acids is studied including minor constituents

isoflavones: daidzein, genistein, glycitein,

- isoflavones are important constituents in soya; all relevant constituents are included; results are expressed as total isoflavones and as aglycon equivalents

As far as human nutrition is concerned validated methods are used, for the assessment of constituents with the exception of the fibre analysis. The "carbohydrate by difference" approach is not very appropriate as it gives no information on the composition of the carbohydrate fraction.

No significant differences were found with the exception of raffinose en oleic acid. The values are however within the range of literature data.

The applicant concludes that the submitted soybean is compositionally and nutritionally equivalent to the parent soybean and to other commercial soybeans.
I agree with this conclusion.

Remark SBB:
For consistency with comments placed for previous applications we propose to place the following comment on the EFSAnet:
Even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document (OECD, 2001), it lacks the analysis on dietary fibre while this concept is widely accepted in human food studies.

Comment of the coordinator
However, again comparisons were made with the literature ranges which are very large. The first question we sould have to answer is to know if the GM event and, in a second time the GM + the herbicide treatment required for that GM, induce differences with the reference line. In that case the answer is yes!
The second question is to know if these differences could have a toxic or a public health concern. Here the answer is probably no because, variations are kept in a range of variations recorded for other varieties that are commercially available. However, it remains that the nutritional value is not equivalent and this could have probably an impact for some uses.

Comment 4

The information provided is sufficient.
Comment: tables 17 to 21 “Results of the by-site t-tests” are not the most convenient way to summarize the results of the statistical evaluation for the components analyzed in raw soybean seeds.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

Clear. No questions.

Comment 2

The information provided is sufficient.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

It is not always clear where the reported data came from. For instance, for the PAT protein content in %dm of crude protein of soybean meal a value of 1.20x10exp-5 is given in the authorisation request for soybean A5547-127 with reference to the Bayer CropScience report of Shillito 2003. In that report (BK99B015F, page 14) a value of 0.000013 is given for meal deriving from transformed soybean A5547-127 when sprayed, and of 0.000019 when not-sprayed. In another Bayer CropScience report (07 B 005 page 63 ; Oberdorfer 2008) the value for this item is 1.34x10exp-5. with reference to the same Shillito report. Although these values are close together, the value reported in the authorisation request for soybean A5547-12 seems not to correspond with the values in the publications referred to.

Comment 2

When comparing Table 33 and Table 34, the non-transgenic soybean shows an increase in lectins, although heat treatment should decrease this value. Although this is not a problem directly related to this transgenic dossier, it poses questions related to the experimental setup and the correct processing conditions during the experiment.
The dossier would be more convincing if these unexpected experimental results are more extensively discussed and/or are repeated.
Furthermore, in the Appendix of Oberdoerfer (2008), there are no details for this experiment about the number of analyses. It is only mentioned that “Lectin results are mean values from several analyses”.

In Table 35, protein content of soy isolate is presented. Although almost all values are within the reference ranges, it is not clear whether the total protein content for the transgenic soybean is significantly lower as compared to the non-transgenic version. Also the original data from study Bk07Q003 (Haas, 2007) are not available for evaluation.

Comment 3

The submitted soybean was processed according to usually applied processing. These include a separation into hulls, non toasted meal, toasted meal, crude oil, refined oil, soybean isolate and lecithin.
If relevant the composition of the fractions was assessed as well for the presence of PAT protein.

The composition of the fractions was comparable to those from the parent soybean. Results were also compared with literature data.
Additional information is given about tocopherols: alpha-, beta-, gamma- en delta-tocopherol. Soybean contains indeed important quantities of other tocopherols than alpha-tocopherol. They are particularly important as natural antioxidants.
The lecithin fraction, a widely used emulsifier, was studied in detail. Individual levels of phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatitidylinositol and lyso-phosphatidylcholine are mentioned.

The PAT protein was assessed in all fractions. The presence was demonstrated in the hulls, the untoasted meal, the toasted meal and soybean isolate.
The PAT protein was not detected in fractions obtained after high temperature heating, screw pressing, solvent extraction. Alkali refining as well as deodorisation removed the last traces of PAT protein. The level was < LOQ (limit of quantification) in refined oil and lecithin.

I agree with the conclusion that the composition of the fractions, obtained from both types of soybean, is comparable.
It is however not clear if the PAT protein is removed or denatured during processing.

Comment 4

The information provided is sufficient.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No changes in consumption pattern due to the introduction of the submitted soybean are to be expected.

Comment 3

The information provided is sufficient.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Pat protein measured in A5547-127 soybean (De Wulf and De Pestel, 2007).

Growth stage/ Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean (n)	Range	
V3 leaf	18.40		6.50
V8 leaf	26.22		9.87
V3 stem	39.18		3.04
V8 stem	13.85		6.12
V3 root	8.16		2.50
V8 root	3.60		0.42
grain sprayed	0.017471		
grain not sprayed	0.020202		

Please provide data based on dry weight.
No standard deviation is present for the grain.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

PAT protein is present in protein isolates from transgenic soybean A5547-127 (Technical report , part I, Table 39). Soy protein concentrates are incorporated in some infant formulas (Bathia et al., 2008) and digestion of proteins in new-borns is limited (Pierce et al., 1967; Henderson et al., 2001). Thus the in vivo digestive capacity in new-borns may be less effective than that found in vitro with simulated gastric and intestinal fluid. **Q.: Are data available on the safety of PAT protein for new-borns?**

Remark SBB and coordinator
This kind of comment has already been transmitted for previous dossiers (RX-MON89788 and UK/2007/43). It remains worthy to insist again, Soya is more and more used in baby food !

Comment 2

a) Degradation of the pat protein in simulated gastric fluid (Rouquie, 2005).

Results confirm the **rapid degradation - within 30 seconds - of the PAT protein in simulated gastric fluid** (SGF), in the presence of pepsin, at pH 2,0.

b) Degradation of the pat protein in simulated intestinal fluid (Esdaile, 2004).

Results obtained with a similar method coupled with a Western blot show the almost **immediate degradation of the PAT protein in simulated intestinal fluids** (SIF) (pH 7.5), in the presence of pancreatin. The residual fragments, at about 5 to 14 kDal, completely disappeared in less than 30 seconds of incubation and were not detectable even with the very sensitive Western Blot method.

c) Pat: Acute Intravenous Toxicity Study in Mice (Kennel, 2003).

The results showed that the animals treated with the PAT protein at 10 mg/kg had no visible signs of systemic toxicity.

7d) Pat: 14-day Oral Toxicity Study in Rats (Pfister *et al.*, 1999).

In conclusion, feeding the PAT protein to rats for 14 days revealed no indications for adverse effects up to the highest dose tested.

The doses used in this study seem to be rather low. How were these amounts chosen?

7e) Pat: Amino acid sequence homology with known toxins (Capt, 2007c)

The overall homology search with the PAT protein, showed no evidence for any similarity to known toxins. **The PAT protein has only high similarity with other non-toxic proteins** (other

acetyltransferase proteins from various origins. No records were found on potential hazard associated with this family of proteins).

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

The aim of the genetic modification is to introduce tolerance to glufosinate by metabolizing it. Thus glufosinate metabolites are expected to be present in soy A5547-127. It has been shown that the composition of the metabolites of glufosinate are different in transgenic plants with the *pat*-gene than in their conventional counterparts (Droge-Laser et al., 1994; Müller et al., 2001). Thus, in my opinion, they can not be considered as residues but rather as plant metabolites.
Q: Are metabolites of glufosinate ammonium present in seed meal and if so, has their toxicological profile been determined?

Comment 2

See above

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Significant differences were found between the octadecenoic acid content (Part I, page 71,Table 22, of glufosinate treated GM soy and control soy. In the discussion of this finding (Part I, page 72), and elsewhere (e.g. Table 36) octadecenoic acid is considered to be oleic acid. This is indeed the major octadecenoic acid in soy but cis-vaccenic acid is present as well in concentrations far above the detection limit (e.g. Baylin et al., 2007). It would be interesting to differentiate these 2 positional isomers to determine whether the difference is mainly due to one of the isomers in order to judge whether there is compositional equivalence. As cis-vaccenic acid is present in human and animal diets, this may not pose a health hazard. **Q: Why is the cis-vaccenic acid content not reported as this fatty acid is easily separated from oleic acid and quantified by modern analytical technology and thus probably available to the applicant?**

Comment 2

See above

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

a) 42-day feeding study with broiler chickens (Leeson, 1998).

There was no effect of starter (day 1-17), grower (day 18-31) and finisher (day 32-42) diets on body weight, body weight gain, feed intake, feed intake:body weight gain or percent mortality over the experimental period (P >0.05).
Carcass characteristics both measured and calculated were unaffected by source of soybeans in the experimental diets.

b) 90-day rat feeding study (author).

Not performed. **No further testing is needed**.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

For section 7.9.2, the experiment to test the allergenicity of the whole GM plant is discussed, but the results are not described in the technical dossier. Although this information can be extracted from Lehrer (1997), it would be easier for the evaluation if this is described in the technical dossier as well.

Comment 3

Assessment of the allergenicity of the newly expressed proteins.
Agreed with the statement that, with the current knowledge, PAT is unlikely to be allergenic.
Assessment of the allergenicity of the whole GM plant or crop.
The applicant did assess the allergenicity of the whole GM plant. The data are supportive of no difference in allergenicity between parental and modified crops. The study, however, is somewhat "old" and one point arose: for RAST inhibition, the reaction to the parental soybean was inhibited by the parental or the modified soybean. It would have been more appropriate to inhibit the reaction to the modified soybean by the parental and the modified. Indeed, if new IgE specificities appear in the

modified soybean, they cannot be put in evidence by inhibiting the parental extract by the modified extract.
The importance and the incidence of soy allergy deserve the reproduction of these experiments with more current techniques and by taking the point above into account.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

In section 7.10, an experiment with broiler chicken is described, but the results are only described in general terms. Evaluation is easier if a summarizing table based on Leeson (1998) is presented in the technical dossier as well.

Comment 3

The information provided is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

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)

Comment 1

The information provided is sufficient.
Comment: The cultivation of A5547-127 soybeans is not included in the scope, the environmental exposure will be restricted to accidental release. Soybean imports in Belgium are unloaded in Antwerp or Ghent where the crushing facilities are situated. Volunteer plants, should they emerge in an area due to spillage from transport, can be destroyed either mechanically or through the use of a herbicide other than glufosinate ammonium.

Comment 2

Soybeans are typically domesticated crop plants that need human intervention for long-term survival (lack of seed dormancy; low spontaneous dehiscence of seedpods, *etc*.). This will not be changed by the introduced herbicide resistance trait.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.
Comments : any plants that might germinate from an accidental spill during import or transport of A5547-127, have no selective advantage over conventionally developed soybeans.

Comment 2

The introduced herbicide resistance trait only provides a selective advantage when the relevant herbicides are applied.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

Comment 2

Other soybean plants are the only possible partners for crossing. As the material will not be cultured in Europe and the structure and function of the flowers was not changed by the genetic modification this is very unlikely. The hybrids would only survive to the next generation if the hybrid seeds are used for further propagation.

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)

Comment 1

The information provided is sufficient.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

See 7.8.1.

Comment 2

The information provided is sufficient.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No extra information required.

Comment 2

The information provided is sufficient.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

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 information provided is sufficient.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided is sufficient.

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