Bioveiligheidsraad
Conseil de Biosécurité

23-09-2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2011_0897

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/UK/2007/43 from Pioneer under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2007/43 was submitted by Pioneer on 11 April 2007 for the marketing of genetically modified soybean 356043 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean 356043 expresses the *gat4601* gene which confers tolerance to the herbicide glyphosate and the *gm-hra* gene that confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron or sulfonylureas.

The application was officially acknowledged by EFSA on 28 September 2007. 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 Annex II for the list of comments actually placed on the EFSAnet on 20 December 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 6 July 2011 (EFSA Journal, 2011;9(7):2310)[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 1 August 2011 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. In addition, the complementary information regarding compositional analysis sent by the applicant to EFSA in the course of the evaluation of the application was provided to the coordinator and to the expert 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/2310.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

3.1. Assessment of compositional analysis

The comparative compositional analysis has shown that the 356043 soybean has significantly higher amounts of N-acetylaspartate (NAA) and N-acetylglutamate (NAG) than its conventional counterpart and commercial soybean varieties. This is a consequence of the incorporation of the GAT4601 protein, which with a low specificity also acetylates aspartic acid and glutamic acid.

Additionally it was found that the 356043 soybean contains significantly higher levels of heptadecanoic and heptadecenoic acid when compared with its conventional counterpart. This is estimated to be an unintended effect resulting from the genetic modification.

Animals fed NAA and NAG did not show any negative effect.

The compositional analysis as performed by the applicant, has not included the analysis of phosphatides in lecithin, as recommended by the OECD consensus document on compositional considerations for new varieties of soybean.

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 document accordingly.

3.2. Assessment of toxicity

See point 3.1.

[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

3.3. 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.

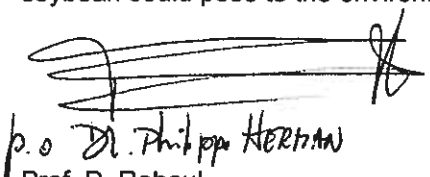
4. Monitoring

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

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council is of the opinion that OECD recommendation regarding the comparative compositional analysis has not been completely followed. The lack of information does not allow the BAC to give an advice on the health safety of the event.

The Biosafety Council did not identify any risk that the import and processing of this GM soybean could pose to the environment.



p.o Dr. Philippe Herman
Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

UK/2007/43

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/~~NL/2008/52~~ *(ref. BAC_2007_PT_619*
Annex II: Comments submitted on the EFSAnet (ref. BAC_2007_PT_624)

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14-12-2007

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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2007/43

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 5 October 2007
Coordinator: René Custers
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Johan Claes (KH Kempen), Eddy Decuypere (KUL), Jean-Claude Grégoire (ULB), Jean-Pierre Hernalsteens (VUB), Peter Smet (Consultant), Nancy Terryn (UGent)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, human nutrition, animal nutrition, biochemistry of food/feed, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, agronomy, herbicide tolerance, ecology, plant-insect relations, bio-diversity, risk analysis, post-release-monitoring, soybean
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/43** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **soybean 356043** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 28 September 2007.
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.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Pioneer Hi-Bred 356043 soybean is tolerant to glyphosate, the active component in Roundup blocking the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and is also tolerant to the ALS-inhibiting herbicides (cholimuron and thifensulfuron), inhibiting the first step in the biosynthesis of branched amino acids (leucine and valine, by inhibiting synthesis of acetolactate from 2pyruvate molecules, and isoleucine by inhibiting synthesis of acetohydroxybutyrate from pyruvate + ketobutyrate).
The tolerance to both groups of herbicides is realized by the expression of 2 new proteins, GAT4601, a glyphosate acetyltransferase (encoded by an optimized form of the gat gene from B-licheniformis), that acetylates glyphosate to the non-phytotoxic N-acetylglyphosate, and GM-HRA-protein, an acetolactate synthase enzyme from the ALS-family of enzymes, conferring tolerance to ALS-inhibiting herbicides.

Comment 2

In general I found the main text of the technical dossier rather difficult to read. There were many references to the figures and tables which had to be looked for at the end of the document.

- Pag 8 of Part III Cartagena: typo: The mortality, body weight gain and feed conversion of the chickens fed with this maize were compared (should be this soybean)
- Part V sampling, there is no confirmation (no signature) that JRC has received these samples, I saw this in other dossier so I guess it is needed?
- Part VI "When available, a validation report of the event specific quantitative detection of 356043 soybean will be published by the JRC-CRL" I guess this is needed before commercialization can be approved? But there is an annex 32 that gives the protocol so it is there?

Note from the SBB:
- The completeness of the dossier (including sample for JRC) is under responsibility of EFSA. The completeness check has been performed and the dossier was declared valid on 28 September 2007.
- The applicant proposes a method. The validation of the event specific detection method is under responsibility of JRC.

Comment 3

No comment/question
NB – My competence is in the environmental effects of GM plants; therefore my contribution in this dossier will be limited to matters within this competence.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

It is noted (p 6) that overwintering is rare, especially in commercial cultivars. How much is "rare" and to which extent could exceptions occur, provided there is any spillage during transportation or processing ?

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

All clear

Comment 3

Sufficient and clear information is provided on the genetic modification.

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

GAT4601 is conferring tolerance to glyphosate by acetylating and as a consequence inactivating glyphosate. This acetylation is not limited to glyphosate however (see further) and one has to be aware of acetylated by-products.
GM-HRA is conferring tolerance to ALS-inhibiting herbicides; since this tolerance is important for the synthesis of leucine, valine, isoleucine when applying ALS-inhibiting herbicides, the question will be

if this tolerance is partial or complete, hence evaluation of levels of branched amino acids in these transformed plants after herbicide treatment will be important.

Comment 2

No comments/questions

Comment 3

The introduced coding sequences and their regulatory elements are clearly described.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

Comment 3

Although adequate techniques were used to analyse the transformation event and the insert, the chapter was not so easy to follow. Relation between text, figures of the Southern blot and the corresponding table 3 were not clear at first. I felt difficult to link all together.

The XbaI Southern story to my opinion was more complex presented than needed. First I am not clear why on figure 9 the sizes of the internal XbaI fragments are indicated twice, one the correct one, the other what is seen in some Southern blots. I would have only mentioned the correct one as they come from the sequence itself. The size indicated as seen on the Southern is 1480 bp for the 5' end, but on all gels I would say the band is migrating somewhat lower then the 1480 bp marker, so this could in fact be the 1379bp band, as indeed is proven in the study in Annex3. It would have been easier for the reader just to mention the Annex3 data without the whole plasmid control Dam-/Dam+ discussion.

Minor remark: In the text and Southern of the als terminator probe (fig 11) there is a difference on the size of the 3' border fragment. Fig 9 it says 750, in the text 800 and on the figure it is actually not clear, as he size of the lower marker, although clearly visible, and just below the border band, is not marked.

Comment 4

Complete information is provided to prove the insertion of a single full length insert, carrying two herbicide resistance genes in the nuclear genome of the transgenic line. These are expressed under the control of a constitutive promoters and confer respectively resistance to glyphosate and ALS-

inhibiting herbicides. This is confirmed by high quality scientific data. The information allows the event-specific detection of this transgenic line.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Protein levels in grain of 356043 are:
- 0.24-0.28 ng/mg dry weight for GAT4601
- 0.46-0.91 ng/mg dry weight for GM-HRA

and no fusion proteins.

Comment 2

No comments/questions

Comment 3

In point 3 there is the paragraph under the heading "expression of new fusion proteins". I guess this is the text as provided by the standard application but in light of new developments in the field of small active peptides and active small RNA's maybe this should cover also that part. I am not sure myself to what degree this study should be done, as the costs for a regulatory dossier are already high, but I have seen in other dossiers a full analysis and even expression analysis of the border region, so maybe there should be some uniform guidelines by EFSA on this.

Comment 4

Constitutive expression of the transgenes, as expected by the nature of the promoters used to express these transgenes was proven by ELISA. No fusion proteins and only one very short new open reading frame are created as a result of the insertion.

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

In North as well as South America agronomic trials comparing 356043 soybean with the Jack soybean with comparable genetic background, it was found that plant height was shorter in the GM-plants but remained within the respective tolerance intervals. No further possible biological reason or hypothesis is given for this possibility of length difference. On a per location analysis, only 1 out of 4 in N.A. and 1 out of 6 in S.A. was statistically significant, and together with the tolerance intervals it was considered as no longer significantly different. However, although this may have no consequences as

for safety of the GM-soybean, the fact that it occurred both in N. & S. America in the same direction still draws some doubts about the biological irrelevance of these possible difference in height between GM and non-GM soybean.

Comment 2

No comments/questions

Comment 3

When no herbicide selection is applied, no statistically significant differences are expected between the behaviour of the transgenic soybean and its non-transgenic parental line. This was confirmed by comparing carefully the phenotype and agronomic traits of both.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

Comment 3

The stability of the insert was proven by Southern DNA hybridisation on hemizygous plants. The stable expression and Mendelian inheritance of the glyphosate resistance was independently confirmed by Western blotting.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

Comment 3

In principle, considering that there is no other species sexually compatible with *Glycine max* in the EU, this question is not relevant here.

Comment 4

I agree that transfer of transgenes from transgenic plants to soil microorganisms is unlikely. To the best of my knowledge I am not aware of a well-documented example. In addition, as no antibiotics resistance genes are involved, it would most likely not have significant consequences. Crossing with other soybeans is also unlikely, because the soybean is mainly self-pollinating and the transgenic line will not be cultured in the European Union.

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 (toxicologic, 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. These results indicate that soybean 356043 will not raise any additional problems for human or animal health as compared to control soybean.

One issue is not properly discussed, as indicated in section D.7.3 of this evaluation.

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

The fractions of several fatty acids in the oil derived from 356043 soybean are significantly different from that of its comparator Jack. These small differences have no impact on health and are well in the literature range of other soybean oils but may possibly point to unexpected effects of the genetic modification. Indeed, these differences can not be due to the effect of herbicide treatment as herbicide treatment or not does not affect the results (Annex 4, comparison of table 10 with table 12).
Q: Are there findings or data which may explain the observed differences?

The levels of N- acetylated glutamate and aspartate in grain are much higher than these of control soybean and values reported in literature (Part I , table 11, page 132). Evidence is given (part 1, page 49) that these findings can be understood in terms of one of the newly introduced traits.
Q.: Can soybean 356043 be considered as substantially equivalent to non genetically modified soybean as obtaining higher levels of these acetylated aminoacids in proteins was not the aim of the genetic modification?

Comment 2

See 7.3.

Comment 3

No comments/questions

Comment 4

No comment/question

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comments/questions

Comment 3

The experimental approach to comparative assessment is well described and the results are convincing.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Selection of materials is logical and the components analysed are adequate.

Comment 2

Because of the nature itself of the genetic modification, particular attention should be given on acetylated products as well as on leucine, valine and isoleucine in GM-soybean combined with herbicide treatment. Separate analysis in N. and S. America may point to biological effects if similar trends are found.
N. America
- No significant differences were found for the branched amino acids leucine, isoleucine and valine.
- No significant differences were found for most of the fatty acids, and if occasional differences (e.g. location dependent) were found, then the values were within the tolerance intervals and combined literature ranges as for oleic, palmitic and linoleic acid.

The use of literature ranges as for tolerance limits may however mask 2 different effects

- a) biological effects, since the genetic background of soybeans analyzed in different plants, different times, seasons… may be different from the control or GM-soybean.
- b) methodological effects, since the analysis in different laboratories over the world may implicate small or large differences in analytical values depending on the analyzed substance.

Both cannot be distinguished when using all available literature data in order to establish tolerance ranges.

- Acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) were significantly higher in 356043 soybean as a consequence of the incorporation of the GAT4601 protein in the GM-plant. However, acetylation is a naturally occurring process in nature as well as in the food industry (e.g. acetylation of L-methionine for supplementation in methionine-deficient feed for animals in order to overcome the Strecker degradation of free L-methionine to methional). Deacetylation is a common process in man and animals, and there is a history of safe consumption of NAA and NAG as well.

It has to be stated that the acetylated products NAA and NAG in 356043 soybean as well as in meal of this soybean is quite high, even in comparison with other commonly consumed goods containing both acetylated products.
However the attention is never drawn on the observation that acetylated products, both NAA and NAG tended to be (or are statistically higher??) higher in treated whole 356043 soybean or soybean meal or hulls compared with herbicide untreated 356043 soybean (see table 19 from part I).
Since glyphosate will compete with aspartate and glutamate for acetylation with the GAT 4601 protein, one should rather expect the reverse! How comes? Is there a logical explanation?

- For Mg, ViE B1 and α-tocopherol, some differences were found, but values remained within the tolerance interval and combined literature range: see my earlier remark on this, although it may not be relevant as for safety use.

South America
In general same trends, hence same remarks as made for N. America.

As for N. America, here as well the range of values for heptadecanoic acid and heptadecenoic acid were higher for 356043 soybean and even higher than the upper end of their respective tolerance intervals and literature ranges.
This is not further explored why? Is there any reason for this that it could be provoked by the 2 introduced proteins? Even if each of these acids represent less than 0.5% of the total fatty acid content of soybeans, and that they are typical constituents of the human diet and can be safely consumed and readily metabolized by humans and animals, it would be worthwhile to know more about the reasons for their increase in 356043 soybean.

Comment 3

The modified soybean increases the amount of two odd chain fatty acids and of two acetylated amino acids. The effect of this increase on possible health effects is well motivated for both types of components (including reference values from literature). In addition, for the acetylated amino acids it is indicated which is the (possible) pathway for the formation of these products, related to the introduced traits (i.e., the GAT enzymes have a known ability to acetylate glutamate and aspartate).
However, for the fatty acids, it is not clear what might be the biochemical explanation for this increase (which is approx. 3 times higher as compared to control soybean). It might be possible that this increase is linked to an increase of other (possibly toxic) components (see, e.g., 2-ketobutyate in

Kingsbury *et al*, 2006; LaRossa *et al.*, 1987). Another link is illustrated by Bjelk and Monaco (1992) who discussed the impact of the herbicide chlorimuron on the fatty acid biosynthesis.

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 (see, e.g., van der Hoeven and Steffens, 2000).

Comment 4

Although this is not my field of expertise, as an evaluator I do have some concerns with the high levels of NAA and NAG present. They are much higher, especially for NAA, than the foodstuff like yeast and meat that the submitters point to as also having these compounds in high amounts.
Table 18, the autolysed yeast gives a high number for NAG, but I guess we never consume large quantities of this as such? In bread it will be way lower.
I am aware that in oil and protein concentrate, the 2 most common products from soybean this is not an issue, but other soybean products like mentioned in table 21 like flour the level is still much higher than known in common food. Is this flour also used for food?
Therefore I do not feel comfortable with the p30 conclusion: "In conclusion, NAA and NAG are normal components of human diets, based on their presence in common foods. There is no evidence to indicate that oral exposure to either NAA or NAG from these sources is associated with adverse effects in humans."

Comment 5

No comment/question

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

Comment 3

No comment/question

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comments/questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Under 7.7 it is stated that the 356043 soybeans and their products "are expected to replace a portion of similar products… ". This "portion" is not specified which is indeed hard to do. Yet, under 7.10.1 it is stated "taking into account the anticipated dietary intake of 356043 soybean products …". This seems to be a contradiction and the latter statement misleading.
Q. : What is the basis for the statement about the anticipated dietary intake of 356043 soybean products? (part II, page 15**)**

Comment 2

No questions

Comment 3

No comments/questions

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

No indications for additional toxicity in Pioneer Hi-Bred 356043 soybean; same level of antinutritional factors as in traditional non-GM soybean.

Comment 2

No comments/questions

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

None of the similar proteins known were identified as toxins, no acute oral mouse toxicity found, no repeated dose dietary toxicity in mice for GAT4601 protein, although 3 clinical chemistry parameters were different between mice consuming control and test diets (containing the purified GAT4601 protein) namely K, total protein and albumin.
However since this was observed for one of the sex groups only, without a dose-response relationship and the magnitudes of the differences being very small, and with no evidence of adverse effects, it was concluded that the observation was not relevant as to adverse effects.

Comment 2

The GAT4601 protein

Equivalency assessment of the GAT4601 protein derived from a microbial expression system with the GAT4601 protein derived from soybeans containing event DP-356Ø43-5 (Comstock, 2006).
The results of the study performed by Comstock indicate that the GAT4601 protein derived from a microbial expression system was equivalent to the GAT4601 protein derived from 356043 soybean leaf tissue and thus the GAT4601 protein derived from a microbial expression system is appropriate for utilization in safety assessment studies as a proxy for the GAT4601 contained in soybean plants.

Acute Oral Toxicity Study of glyphosate acetyltransferase (GAT) 4601 protein in Mice **(**Finlay, 2006).
A single dose of GAT 4601 test substance (containing at least 95% GAT 4601 protein) was administered by oral gavage to groups of 5 fasted male and 5 fasted female Crl:CD®-1(ICR)BR mice at a target dose of 2000 mg/kg. Control groups of 5 fasted male and 5 fasted female mice were administered Bovine Serum Albumin at a target dose of 2000 mg/kg, or vehicle alone, once by oral gavage. The mice were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The mice were sacrificed and given a complete gross pathology examination to detect grossly observable evidence of organ or tissue damage or dysfunction.

All mice survived until the scheduled sacrifice on Day 14. No clinical signs of systemic toxicity or test substance-related body weight losses were observed in any mice. No gross lesions were observed in the mice at necropsy.

Under the conditions of this study, administration of recombinant protein GAT 4601 to male and female mice at a target dose of 2000 mg/kg produced no test substance-related clinical signs of toxicity, body weight losses, gross lesions, or mortality.

28-Day toxicity study of glyphosate acetyltransferase (GAT) 4601 protein administered by diet to CD-1 mice (Babb, 2007)

Consumed dosages for males averaged 7.794, 76.727, and 783.09 mg/kg/day for Groups 2-4 with target doses of 10, 100, and 1000 mg/kg, respectively. Consumed dosages for females averaged 9.183, 94.349, and 926.897 mg/kg/day for Groups 2-4 with target doses of 10, 100, and 1000 mg/kg/day, respectively.

There were no clinical observations or changes in body weight, feed consumption, ophthalmology, hematology, organ weights, or gross or microscopic pathology that were considered test article-related. There were a few statistical differences in clinical chemistry parameters, but none were considered to be clearly test article-related.

In conclusion, ingestion of glyphosate acetyltransferase (GAT) 4601 protein by mice for 27 consecutive days resulted in no remarkable findings of toxicity.

The GM-HRA protein

Equivalency assessment of the GM-HRA protein derived from a microbial expression system with the GM-HRA protein derived from soybeans containing event DP-356Ø43-5 (Comstock, 2006).

The results of the study indicate that the GM-HRA protein derived from a microbial expression system is equivalent to the GM-HRA protein derived from 356043 soybean leaf tissue and thus the GM-HRA protein derived from a microbial expression system is appropriate for utilization in safety assessment studies as a proxy for the GM-HRA protein contained in soybean plants.

Acute Oral Toxicity Study of GM-HRA protein in Mice (Finlay, 2006)

A single dose of GM-HRA test substance in water was administered by oral gavage to groups of 5 fasted male and 5 fasted female Crl:CD(ICR) mice at a dose of 2000 mg/kg. This corresponded to a per-animal exposure of at least 436, but less than 582, mg/kg recombinant GM-HRA protein. Two control groups, each consisting of 5 fasted male and 5 fasted female mice, were administered Bovine Serum Albumin at a dose of 2000 mg/kg in water, or vehicle (water) alone, once by oral gavage. The mice were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The mice were sacrificed and given a complete gross pathology examination to detect grossly observable evidence of organ or tissue damage or dysfunction.

All mice survived until the scheduled sacrifice on Day 14. No clinical signs of systemic toxicity or test substance-related body weight losses were observed in any mice. No gross lesions were present in the mice at necropsy.

Under the conditions of this study, administration of recombinant GM-HRA test substance to male and female mice at a dose of 2000 mg/kg produced no test substance-related clinical signs of toxicity, body weight losses, gross lesions, or mortality.

Comment 3

No comments/questions

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Not applicable

Comment 2

No comments/questions

Comment 3

The applicants state: "Not applicable as the genetic modification in 356043 soybean does not give rise to the expression of any new constituents other than the GAT4601 and GM-HRA proteins." I feel like they cannot conclude this. Maybe the GAT enzyme acetylates other yet unknown compounds in the plant, which could thus form a new constituent. Again this is not my field of expertise but something as a scientist and consumer wonder about. But then again the field studies and animal feeding test might have shown unwanted events here.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

It is claimed that N-acetylaspartate and N-acetylglutamate are normal constituents of the diet and this is given as one of the arguments that these components are safe. However, from the data given (part I, page 145 and page 148), the level of these components in soy 356043 seems to be much higher than in other foods**.**
Q.: Are there data available to estimate dietary intake of N-acetylaspartate and N-acetylglutamate by non-soybean 356040 in humans and animals? To which amount of intake of soybean 356040 products would that account?

Comment 2

See also 7.3., discussion and questions

- about heptadecanoic and heptadecenoic acid
- about N-acetylglaspartate (NAA) and N-acetylglutamate (NAG)
- about differences in NAA and NAG in 356043 soybean with or without herbicide treatment.

Comment 3

No comments/questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

In spite of compositional differences, no effects as for the nutritional value for non GM versus 356043 soybean in the broiler trial was observed. Therefore nutritional equivalency.

Comment 2

<u>42-day poultry feeding study (Delaney *et al.*, 2006)</u>

The objective of this study was to evaluate the nutritional equivalence of 356043 soybean by comparing growth performance and carcass yield of broiler chickens fed diets containing processed fractions (meal, hulls, and oil) from 356043 soybean with those fed diets produced with processed fractions from non-transgenic soybean. Two lots of 356043 soybean were used: the first lot was produced from plants that received no herbicide treatment (356043) and the second lot was from plants treated with a mixture of glyphosate, chlorimuron, and thifensulfuron (356043+Gly/SU). Diets produced with soybean fractions from non-transgenic near-isoline (Control), 356043, 356043+Gly/SU, and non-transgenic commercial varieties (93B86, 93B15, and 93M40) were fed to Ross x Cobb broilers (n = 120/group, 50% male and 50% female) for a period of 42 days.

No statistically significant differences were observed in mortality, weight gain, feed efficiency (corrected for mortalities), and carcass yields between broilers consuming diets produced with 356043 or 356043+Gly/SU soybean fractions and those consuming diets produced with near isoline Control soybean fractions. Additionally, all response variables evaluated in Control, 356043, and 356043+Gly/SU groups fell within the tolerance intervals of the values observed in broilers fed diets produced with the reference soybean fractions.
Based on the results from this study, it was concluded that 356043 soybean was nutritionally equivalent to non-transgenic control soybean with a comparable genetic background.

<u>13-Week feeding study in rats.</u>

It is recommended to perform such a study since synergistic effects of the proteins under investigation cannot be excluded beforehand. Furthermore, it provides insight into longer-term effects of both proteins in mammalian species.

Comment 3

No comments/questions

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Neither the protein GAT4601 nor GM-HRA protein show any significant sequence identity with known allergens, whatever a threshold of sequence identity of 35% may mean.
Both proteins are rapidly degraded in simulated gastric (30 seconds) and intestinal fluids (1 minute for GM-HRA and 2 minutes for GAT4601).
Expression of GAT4601 and GM-HRA in 356043 soybean does not alter the allergenic potential of soybean.

Comment 2

No comments/questions

Comment 3

Assessment of allergenicity of the introduced traits.
The reviewer agrees with the conclusion of the applicant when it is said that GAT4601 and GM-HRA are not likely to be allergenic proteins.

Assessment of allergenicity of the whole GM plant.
The applicant has attempted to determine the allergenicity of the whole transgenic crop with the right methods. However, the number of soybean-sensitive sera is too limited. It is recommended that at least 20 sera be used, in order to get a broader range of reactivity patterns. In addition, the sera should not be pooled, as some information (for example the visualisation of a new allergen) might be diluted and lost in a pool. Whether the allergenicity of 356043 soybean is similar to that of control Jack soybean remains undecided.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

There seems to be no problem.

Comment 2

No questions, nutritional equivalency proven.

Comment 3

No comments/questions

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

It is mentioned that "post-market monitoring of GM food/feed products derived from 356043 soybean is not necessary". I think it is better to state, as in the monitoring plan in fact that general surveillance will be done for 10 years as the monitoring is not based on a particular hypothesis but it should be used to identify the occurrence of unanticipated adverse effects of the viable GMO or its use for human and animal health or the environment that were not predicted in the e.r.a.

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

Comment 1

Not applicable.

Comment 2

No comment/question

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

No questions.

Comment 2

The lack of weediness traits, as described on pp. 57-8 is convincing. Treating the GM seeds as any commercial soybean might results in seed spillage but unwanted dissemination is not likely.

Comment 3

Persistence and invasiveness is not a problem with any soybean. No wild *Glycine max* is known and there are no examples of soybeans that survive in natural environments and become weeds.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Not likely here.

Comment 3

The presence of the herbicide resistance genes should only give a selective advantage to the plant when the corresponding herbicides are applied.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Quite unlikely, as there is no sexually compatible relatives in the EU.

Comment 3

There are no plants in the European wild flora that exchange genes by hybridisation with soybeans. In addition, the culture of this soybean line in Europe is not intended. Therefore no impact of this plant on the European environment is expected.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not applicable.

Comment 2

Not relevant (no target organisms).

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Not likely

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

An unusual fatty acid, designated as "linoleic acid isomer (9,15)" (probably 9 cis, 15 cis octadecadienoic acid is meant) has been reported in the oil from soy 356043 in the South America study at very low levels. In connection with its safety, it may be important to note that this fatty acid has also been found in control soybean (Part I, table 13, page 138) and in mango (Shibahara etal., 1993) and thus may be a normal constituent of the diet. Moreover it has been described to be present in the rumen of ruminants (Loor et al., 2002). Several minor polyenoic fatty acids have also been described in ruminant fat (Alves et al., 2007) without posing a health problem.

The scope of the application of the the genetically modified crop is for all food and feed uses. One of the uses of soy protein is in baby formula. It has been shown that N-acetylaspartate and N-acetylglutamate were much higher in soybean 356043 but below the limit of detection in protein isolate. The protein isolate was obtained using "standard" processing procedures.
New procedures to obtain protein isolate which are claimed to be cheaper and not to denaturate the proteins are being planned (for canola) (Anonymous, 2007). Thus it is not clear that all protein isolates in the future will be be virtually free from N-acetylaspartate and N-acetylglutamate when Soybean 356043 is used. This may pose a problem for new-borns who have low proteolytic activity (Henderson etal., 2001) and if absorbed intact may or may not be catabolised efficiently intracellularly (Perrier et

al., 2005). At any rate, N-acetylaminoacids amongst which acetylaspartic (Gerlo et al, 2008 ; Kvittingen et al., 1986) acid have been found in elevated levels in urine of children with disturbed acetylaminoacid metabolism. Although these N-acetylaminoacids are not the cause of these diseases they may possibly contribute to their clinical manifestations. Further note that N-aminoacids have have physiopathological roles in the brain (e.g. Yan et al, 2003).

Q: Will producers of infant food be notified that new methods for preparing soy protein from Soybean 356043 may possibly result in increased levels of N-acetylaspartate and N-acetylglutamate in the protein and that the toxicity of these components in infant formula has not been determined?

Comment 2

No questions.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

Not relevant here.

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

Comments/Questions of the expert(s)

Comment 1

Not applicable.

Comment 2

Not relevant here.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No potential impact of Pioneer Hi-Bred 356043 soybean on biotic or abiotic environment is expected to result from the import, processing or use of this product for food and feed in EU.
If an impact has to be expected or hypothesized, then it could be the effect of glyphosate or ALS-inhibiting herbicides used when 356043 soybean is cultivated, and the widespread use of these herbicides will be made possible and promoted by the use of GM-soybean 356043. However, since this application is for consent to import 356043 soybean and products in EU and to use it as any other soybean, excluding the cultivation of 356043 soybean, it also excludes the usage of mentioned herbicides, hence its potential impact on biotic or abiotic environment in the EU, but not outside the EU.

Comment from SBB: the metabolism and residues of the herbicides in genetically modified herbicide-tolerant plants are already considered in the regulatory process for herbicide registration or extension of existing registrations which is covered by Directive 91/414/EEC[1].

Comment 2

Not likely

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

The information is satisfactory.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No comments.

[1] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market

Comment 2

No comment/question

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

Not relevant here.

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

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

The methods proposed for general surveillance of 356043 soybean is adequately described.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

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Bioveiligheidsraad
Conseil de Biosécurité

20 December 2007

Secretariaat
Secrétariat

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Application EFSA/GMO/UK/2007/43
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 5 October 2007
Coordinator: René Custers
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Johan Claes (KH Kempen), Eddy Decuypere (KUL), Jean-Claude Grégoire (ULB), Jean-Pierre Hernalsteens (VUB), Peter Smet (Consultant), Nancy Terryn (UGent)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, human nutrition, animal nutrition, biochemistry of food/feed, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, agronomy, herbicide tolerance, ecology, plant-insect relations, bio-diversity, risk analysis, post-release-monitoring, soybean
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/UK/2007/43** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **soybean 356043** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 28 September 2007.
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)

Comments posted on the EFSAnet

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). Below the comments as they were forwarded to the EFSAnet, and in a separate document[1] the compilation of all the comments that were given by the experts (including the references). Only those comments that raised a question or a concern were forwarded to the EFSAnet. The fact that comments that did not raise a question or a concern were not forwarded to the EFSAnet does not diminish the value of these comments. They are absolutely necessary for the complete analysis of the dossier, and will be used in formulating the final advice by the Biosafety Advisory Council.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comments were raised on the set-up of the dossier with many references to the figures and tables which had to be looked for at the end of the document. This set-up makes the main text of the dossier rather difficult to read.
Attention has been drawn to a typo on page 8 of Part III Cartagena: The mortality, body weight gain and feed conversion of the chickens fed with this maize were compared (should be this soybean).

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

Comments/Questions of the expert(s)

The information in this part of the dossier is regarded adequate.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

The information in this part of the dossier is regarded adequate.

[1] Référence: BAC_2007_PT_619

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)

The information in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

It has been remarked that the presentation of the XbaI digest Southern blot was unnecessary complex. In figure 9 of the main dossier the XbaI digest is presented as giving rise to fragments of "~1480 bp/1379 bp" and "~3900 bp/3927 bp". To the opinion of our experts Annex 3 of the dossier proves that the bands are the expected bands of 1379 and 3927 bp. The confusing discussion on Dam-/Dam+ in the main dossier could have been avoided if only the Annex 3 report was mentioned.

A minor remark: In the text of the main dossier and its figure 9 there is a difference on the size of the 3' border fragment generated by EcoR V digestion. Fig 9 says "~750", while in the text on page 17 a "800 bp 3' border band" is mentioned. The Southern itself (fig 11) does not give a decisive answer which of the two numbers is correct as the size of the lower marker is not given.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

In North as well as South America agronomic trials comparing 356043 soybean with the Jack soybean with comparable genetic background, it was found that plant height was shorter in the GM-plants but remained within the respective tolerance intervals. No further possible biological reason or hypothesis is given for this possibility of length difference. On a per location analysis, only 1 out of 4 in N.A. and 1 out of 6 in S.A. was statistically significant, and together with the tolerance intervals it was considered as no longer significantly different. However, although this may have no consequences as for safety of the GM-soybean, the fact that it occurred both in N. & S. America in the same direction still draws some doubts about the biological irrelevance of these possible difference in height between GM and non-GM soybean.

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

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

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)

A number of questions have been raised with regard to the high levels of NAA and NAG and the significant higher levels of two fatty acids (see comments under 7.3).

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

The information given in this section of the dossier is regarded adequate.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Because of the nature of the genetic modification, our experts have paid particular attention to acetylated products as well as on leucine, valine and isoleucine in GM-soybean combined with herbicide treatment. Separate analysis in North and South America may point to biological effects if similar trends are found.

Separate analysis of the results from the component analysis of GM soybeans cultivated in North and South America shows the following:

- There are no significant differences for the branched amino acids leucine, isoleucine and valine.
- Acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) are significantly higher in 356043 soybean as a consequence of the incorporation of the GAT4601

protein in the GM-plant. However, acetylation is a naturally occurring process in nature as well as in the food industry (e.g. acetylation of L-methionine for supplementation in methionine-deficient feed for animals in order to overcome the Strecker degradation of free L-methionine to methional). Deacetylation is a common process in man and animals, and there is a history of safe consumption of NAA and NAG as well.
However, it has to be stated that the level of acetylated products NAA and NAG in 356043 soybean as well as in meal of this soybean is quite high, even in comparison with other commonly consumed goods containing both acetylated products.
In the dossier the attention is never drawn to the observation that the level of acetylated products NAA and NAG tends to be (or is statistically higher?) higher in glyphosate treated 356043 soybean (whole soybean, meal or hulls) compared with glyphosate untreated 356043 soybean (see table 19 from part I). Since glyphosate will compete with aspartate and glutamate for acetylation with the GAT 4601 protein, one should rather expect the reverse. How does this come? Can the applicant provide an explanation for this?

- Both in North and South America the levels of heptadecanoic and heptadecenoic acid were significantly higher in 356043 soybean. In South America the levels were higher than the upper end of their respective tolerance intervals and literature ranges. Our experts question why this is not further explored and are of the opinion that the dossier would be more convincing if a possible biochemical pathway would be discussed/hypothysed, based on literature survey and/or experiments (see, e.g., van der Hoeven and Steffens, 2000). It is regarded as possible that the increase is linked to an increase of other (possibly toxic) components (see, e.g., 2-ketobutyate in Kingsbury *et al*, 2006; LaRossa *et al*., 1987) Another link is illustrated by Bjelk and Monaco (1992) who discussed the impact of the herbicide chlorimuron on the fatty acid biosynthesis.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.7.5 Product specification

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Under 7.7 it is stated that the 356043 soybeans and their products "are expected to replace a portion of similar products… ". This "portion" is not specified which is indeed hard to do. Yet, under 7.10.1 it is stated "taking into account the anticipated dietary intake of 356043 soybean products …". This seems to be a contradiction and the latter statement somewhat misleading. The question is raised what the basis is for this statement about the anticipated dietary intake of 356043 soybean products? (part II, page 15).

D.7.8 Toxicology

Comments/Questions of the expert(s)

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

The applicant states: "Not applicable as the genetic modification in 356043 soybean does not give rise to the expression of any new constituents other than the GAT4601 and GM-HRA proteins." The remark is made that the applicant cannot be completely sure that the GAT protein does not acetylate other compounds than glyphosate, glutamate and aspartate, and in that way generate other new constituents,

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Here the concerns are repeated on the high levels of NAA and NAG. The level of these components in soy 356043 seems to be much higher than in other foods. When one takes this high level into account: Can the fact that NAA and NAG are normal constituents of our diet then be used as a safety argument?

Also the concern on the differences between NAA and NAG levels between herbicide treated and non-treated GM soybean, and the concern on the higher levels of heptadecanoic and heptadecenoic acid are repeated here. The question is also raised whether there are data available to estimate the dietary intake of NAA and NAG by non-GM soybean in humans and animals.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.7.9 Allergenicity

Assessment of allergenicity of the whole GM plant.
The applicant has attempted to determine the allergenicity of the whole transgenic crop with the right methods. However, the number of soybean-sensitive sera is too limited (only five, see annex 30). It is recommended that at least 20 sera be used, in order to get a broader range of reactivity patterns. In addition, the sera should not be pooled, as some information (for example the visualisation of a new allergen) might be diluted and lost in a pool. Because of this poor methodology the question whether the allergenicity of 356043 soybean is similar to that of control Jack soybean remains undecided.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

The remark is made that instead of stating that "post-market monitoring of GM food/feed products derived from 356043 soybean is not necessary", it is better to state, as in fact is done in the monitoring plan, that general surveillance will be done for 10 years as the monitoring is not based on a particular hypothesis. This general surveillance will be used to identify the occurrence of unanticipated adverse effects of the viable GMO or its use for human and animal health or the environment that were not predicted in the environmental risk assessment.

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)

The information given in this part of the dossier is regarded adequate.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

D.9.4 Interactions between the GM plant and target organism

Not applicable.

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

The information provided in this part of the dossier is regarded adequate.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

The scope of the application of the genetically modified crop is for all food and feed uses. One of the uses of soy protein is in baby formula. It has been shown that NAA and NAG were much higher in soybean 356043 but below the limit of detection in protein isolate. The protein isolate was obtained using “standard” processing procedures.
New procedures to obtain protein isolate which are claimed to be cheaper and not to denaturate the proteins are being planned (for canola) (Anonymous,2007). Thus it is not clear that all protein isolates in the future will be virtually free from NAA and NAG when Soybean 356043 is used. This may pose a problem for new-borns who have low proteolytic activity (Henderson et al., 2001) and if absorbed intact may or may not be catabolised efficiently intracellularly (Perrier et al., 2005). At any rate, N-acetylaminoacids amongst which acetylaspartic (Gerlo et al., 2006; Kvittingen et al., 1986) acid have been found in elevated levels in urine of children with disturbed acetylaminoacid metabolism. Although these N-acetylaminoacids are not the cause of these diseases they may possibly contribute to

their clinical manifestations. Further note that N-aminoacids have physiopathological roles in the brain (e.g. Yan et al., 2003).
The question is raised whether producers of infant food will be notified that new methods for preparing soy protein from soybean 356043 may possibly result in increased levels of NAA and NAG in the protein and that the toxicity of these components in infant formula has not been determined.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

D.9.8 Effects on biogeochemical processes

Not relevant.

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

Not relevant here.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

The information given in this part of the dossier is satisfactory.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

The information given in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

The methods proposed for general surveillance of 356043 soybean is adequately described.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

No comments.

References
see document BAC_2007_PT_619 in annex