Biosafety Advisory Council

12-03-2007

Secretariat

O./ref.: WIV-ISP/BAC/2007_SC_461
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Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/NL/2005/13** of Bayer CropScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2005/13 was submitted by Bayer CropScience in March 2005 for the marketing (import and processing) of the glyphosate-tolerant genetically modified LLCotton25 for food and feed applications under Regulation (EC) No. 1829/2003[1]. It has been officially acknowledged by EFSA on 2 September 2005.

On the same date EFSA started the 3 months formal consultation of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms (GMOs) being part of the products). In absence of the necessary resources the Belgian Biosafety Advisory Council didn't take part in this consultation.

However, in early 2006, in order to give advice to our minister the Belgian Biosafety Advisory Council was in the position to contact experts to assist in the evaluation of the dossier. These experts were chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology. Five experts answered positively and assisted in the evaluation. The evaluation took place under the supervision of a coordinator who is a member of the Council.

The comments received from the Belgian experts (see Annex II for an overview of all the comments) are synthesised below by the coordinator.

[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)

The opinion of EFSA's scientific panel on GMOs was adopted on 6 December 2006 (The EFSA Journal, 2006, 429, 1-19)[2]

On 20 December 2006 the opinion of EFSA was forwarded to the Belgian experts. The experts were invited to give comments and to react on the EFSA opinion, and asked specifically, based upon their knowledge of the dossier, whether there are essential points in the dossier have not been taken into account in the opinion of EFSA.

Scientific evaluation

The comments of the experts address two different levels : (i) issues relating to the authorization and the monitoring of the GM plant and (ii) scientific questions about discrepancies or unanswered issues in the dossier that could require an improvement of the procedure. This second set of questions is listed in Annex I of the current document. The Belgian Biosafety Council is of the view that the dossier would have gained in clarity if these questions would have been addressed by the applicant.

Regarding issues related to point (i) above, the main points raised by the Belgian experts can be summarised as follows (for the scientific evaluation we refer to annex II):

1. Molecular characterisation

No issues were raised by the Belgian experts.

2. Food/feed safety assessment

2.1 Toxicological assessment of the whole GM food/feed

No issues were raised by the Belgian experts.

2.2. Nutritional assessment of GM food/feed

No issues were raised by the Belgian experts.
It has been noticed however that Gossypol, a typical anti-nutritive factor in cotton seed, does not seem to result in extra disorders in the case of LLCotton25.

2.3 Allergenic assessment of GM food/feed

No issues were raised by the Belgian experts.

[2] see: http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/858.html

3. Environmental risk assessment and monitoring plan

No major issues were raised by the Belgian experts. However accidental spillage of cotton seeds is unavoidable. If it occurs in southern Europe, it is likely that cotton would establish a feral population. Even if it is less likely that this feral population would be in the vicinity of a commercial cotton field and even less likely that out-crossing would significantly alter overall genetic purity of a commercial field, the Belgian experts recommend the monitoring of eventual feral populations in harbours, transit road-sides and vicinity of processing plants on a yearly basis, South of 42°N (this comment is also put forward by experts from Finland, Germany, Spain, Norway and Austria).

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts,
Taking into account the opinion of EFSA's GMO scientific panel,
The Biosafety Advisory Council:

a) Agrees with the conclusion of the GMO panel of EFSA that: "it is unlikely that LLCotton 25 will have adverse effects on human and animal health or the environment in the context of its proposed uses".

b) Supports the GMO Panel recommendation in line with the proposal of the German experts that "specific measures should be introduced to actively monitor the occurrence of feral cotton plants". This should be done South of 42°N in harbours, transit road-sides and vicinity of processing plants on a yearly basis. The elaboration of these measures should be a prerequisite for the authorization.

Experts pointed out several mistakes and unanswered questions in the dossier without proven impact on the biosafety (see Annex I). On the long term and in order to improve our knowledge on the impact of the GM plants, it would have been interesting if some of these questions would have been addressed by the applicant.

p.o.

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

Annex I: List of scientific questions raised by the Belgian experts about discrepancies or unanswered issues in the dossier

Annex II : Full comments of experts in charge of evaluating application EFSA/GMO/NL/2005/13 (ref: BAC_2007_PT_435)

Annex I: List of scientific questions raised by the Belgian experts about discrepancies or unanswered issues in the dossier

1. Molecular characterisation

- Is the Western Blotting accurate enough to describe the insert without bias?
- Why did the analysis not combine Western Blot and FISH analysis to clarify and double check the organisation of the insert event?

2. Food/feed safety assessment

Nutritional assessment of GM food/feed

- Is it possible to give a detailed list of the anti-nutrients? (Technical dossier, Part I, P.57)
- It would be interesting to mention the references used to give the range of cotton seed components. (P.59, Table 14)
- Why is there no reference range for the different classes of fatty acids? How can it be explained that same fatty acid concentrations are lower in the analysed material in comparison with the reference range, while crude fat is within the range? Why is only vitamin E given in Table 14? (P.60, Table 14)
- Does it mean that the use of transgenic cottonseed in animal diets requires more phosphorus, or more phytase? (P.60, Table 14)
- It may be interesting to have a correct explanation for the higher zinc content in LLcotton25 (P.66). However, the zinc content is far below the maximum tolerable level for domestic animals.
- Is the content of crude protein, crude fat and zinc in LLcotton25 significantly different from Coker312? (Table 20)
- Is the content of crude protein in LLcotton25 significantly different from Coker312? May we assume that differences correspond with $P \leq 0.05$? (Table 21 and 22)

For the full scientific evaluation and the bibliographic references please refer to annex II.

Annex II : Full comments of experts in charge of evaluating application EFSA/GMO/NL/2005/13 (ref: BAC_2007_PT_435)

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Bioveiligheidsraad
Conseil de Biosécurité

March 2006

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT/435[1]
Email. : bac@sbb.ihe.be

Expertise report for the EFSA dossier EFSA/GMO/NL/2005/13 - Compilation of all the comments received from the experts

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 12 december 2005
Coordinator: Prof. Philippe Baret
Experts: Leo Fiems (CLO), Jean-Luc Hofs (CIRA), Wim Stevens (UA), Nancy Terryn (UGent)
Domains of expertise of experts involved: genetics, genome analysis, genetic engineering, agronomy, plant-insect relation, biosafety research, animal nutrition, immunology, alimentary allergology, cotton.
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2005/13** concerns a notification of the company **Bayer CropScience** for the marketing of the genetically modified **LLcotton25** for food and feed applications under Regulation (EC) 1829/2003.
The notification has been officially acknowledged by EFSA on 02 September 2005.
The scope of the application is:
☒ GM plants for food use
☐ Food containing or consisting of GM plants
☐ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☐ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the notification on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. Its was expected that the expert should evaluate if the information provided in the notification is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the 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

[1] revised version of document BAC_2006_PT_334

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

At this stage, information provided is satisfactory regarding requirements.

Comment 2

The dossier has been evaluated from the point of view of the use of cotton seed products in animal nutrition.

Comment 3

In general and for the part on the molecular data that I focussed on I appreciated very much the not too heavy structure of the file, with only the "needed" information and the direct links to the company papers with more details when wished.

Although not my expertise, in the summary part F there is no suggestion for labelling but I assume that there has to be?

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

Comments/Questions of the expert(s)

Comment 1

Page 17, 2a i: I would prefer another definition of cotton reproductive mode: Cotton is a facultative self-pollinator and an opportunistic out-crosser when insects pollinators are present (Oosterhuis & Jernstedt, 1999).
Page 17, 2a ii: Hymenoptera are not the lone pollen vectors of cotton. *Coleoptera* can take a great place in the pollination process (Sanchez et al, 2004) and (Hofs, to be published). Assessment can't be only based on the 3 mentioned *taxa.* Pollen transfer can be largely influenced by the entomological diversity in a place (ecological region, e.g.). The use of insecticides on crops unfortunately reduces a part of the pollinating insect populations in a field. But to what extend? This is very variable and nowadays pest management becomes more "environmental friendly" (even in the US) through the use of IPM programs. That means a better respect for beneficial insects (and among them flower visitors). So, insecticide programs can't be displayed as a barrier (or a break) to insect pollination.
Page 18, 2b: Natural inter-specific crosses between *Gossypium hirsutum* and diploid species are little likely but not impossible. In Australia, Brubaker (An., 2002) observed 3 individual plants produced from *G. sturtianum* and *G. hirsutum* in the field without the application of plant hormones. Production of non reduced gametes is rare but possible with some crosses between *G. hirsutum*, *G. exiguum* and *G. nobile* as it has been mentioned in (Brubaker et al., 1999). This means that sometime a triploid hybrid (generally considered as sterile) produces fertile pollen.

Page 18, 3a: Regarding seedling difficulty to push its way through the soil, it is only an assumption. This incident may occur but, fortunately for cotton farming, in rare cases, generally when the soil is "too hard or capped with a hard layer above the seed" (Munro, 1987).
Page 18, 3b: Regarding the control of volunteers, it is feasible in the farmer's field but not when volunteers become feral outside the field (even in its vicinity) or along roadsides, for instance.
Page 19, 4: There are, of course, physical barriers and others impediments that may reduce the potential for pollen movement but in what proportion? I think there is no accurate answer at this stage.
Page 19, 4a: The applicant is confusing about separation distances and multiplication classes. Since GM plant adoption, Foundation and Registered seeds must be produced under at least 1320 feet (440 m) (USDA) and (Sundstrom et al, 2002). Minimum isolation shall be at least 100 feet (30 m) if the cotton plants in the contaminating source differ by easily observable morphological characteristics from the field to be inspected.
Page 19, 4b: Seed may be transported (for rapid consumption) by rodents or partridges, even in glanded cotton (case in Africa).

Comment 2

Cotton seed varieties can be divided into two classes: low lint and high lint varieties. It is not clear to what class Coker312 is belonging. On the one hand, it is questionable if the parent variety is a good representative of cotton seed on average, as moisture and fat (Table 19) are out of the range, based on literature data. On the other hand, LLcotton25 does not seem to differ considerably from the parent variety.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

No comment.

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)

No comment at this stage.

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

Comments/Questions of the expert(s)

Comment 1

Is the Western Blotting accurate enough to describe the insert without bias?
Why did the analysis not combine Western Blot and FISH analysis to clarify and double check the organisation of the insert event (Walters et al., 1998) and (Zheng et al., 2001)?

Comment 2

As the insert is not so complex and has been completely re-sequenced I have no questions in relation to it. Also the Southern blots, both in the main file and the sited company papers are clear and the conclusions drawn seem correct to me.

D.3. Information on the expression of the insert

Comments/Questions of the expert(s)

No comment.

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

Comments/Questions of the expert(s)

What about the possibility/existence of feral or escaped plant from the field? These plants are uneasy to spot and control.

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

Comments/Questions of the expert(s)

No comment.

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

Comments/Questions of the expert(s)

Page 46, 6b: In South Africa (Hofs, to be published) we found 0.9% of average out-crossing at 25 m from the source but, locally, hybridization rates of 30% at 40 m from the source have been pointed out.

Page 47: Seed number for glufosinate tolerance is relatively low for out-crossing detection and may lead to inaccurate results. One estimates a number of 500 seeds to get reliable results for that purpose.

Page 48, 6b ii: Insect vectors: we do not know if there are no insects that can carry pollen over long distances. What is clear, is that we know some *Coleoptera* in Africa and South America able to move pollen outside the field (Sanchez et al, 2004) and (Scholtz & Holm, 1996). Cotton pollen is viable during 12 hours, in average (Govila & Rao, 1969) and an insect may travel far during such a period. The positive point for biosafety impact is that this pollen escaping will be strongly mitigated into the environment (all individuals of an insect species do not fly in the same direction and converge to the same spot).

Coincidence of flowering: cotton flowering period is long (from 50 to 90 days or more if there is no climatic limitation) (Munro, 1987) and abundant. Two cotton plants planted at different times are very likely to have overlapping flowering periods. In a particular area, coincidence of flowering can't be a criterion for gene flow limitation in cotton (*Gossypium sp*.).

Crossing with wild species: (see B)

Need of human interventions to survive outside the field: this is a "cliché" often pinned on cotton's back. A cotton plant doesn't grow as a weed and will never yield as much as one in a well cared field but is capable of surviving in the bush or along roadsides. Feral cotton populations can survive "in the wild" over years; and that is the case of cotton in the Mpumalanga and KwaZulu Natal

province in South Africa (Hofs, to be published). There is unfortunately no scientific publication on that topic.

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

The company has compared the PAT protein produced by the cotton seed with the protein produced by *E. coli* and did not find differences. Made comparison of the PAT protein sequences in databases of known allergens and did not find striking similarities when sequences of 8 amino acids were compared.

They also deduced that the PAT protein would not be allergenic since it is

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and are present at high concentrations in the food. Data has been submitted which demonstrate that the PAT protein is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. Thus, the potential for the PAT protein to be a food allergen is minimal.
EPA 1997, Federal register 17718

Herouet et al. (2004a C044359.pdf) concluded:
It can also be concluded that the 5-prime flanking sequence does not code for known proteins which have potential toxic or allergenic properties. This conclusion is emphasised by the fact that this flanking sequence does not represent an integration of the gene cassette in a coding region of the wild-type genome. Moreover, the phenotypic analyses support this conclusion by showing no alteration of the fibre quality (reports #C023704 and #C023705).

The safety and lack of allergenicity was further evaluated by Herouet et al 2004c (C045036.pdf)

This safety evaluation includes both a review of published literature and of recent studies using the PAT protein produced by the *bar* gene for: a) amino acid homology comparisons to known toxins and allergens; b) potential glycosylation consensus sequence comparison; c) protein stability to heat and in digestive fluids; and d) acute toxicity by intravenous injection in the mouse.

The assessment supports the following conclusions:

- The donor organism (*Streptomyces hygroscopicus*), which contains the *bar* gene, is a common soil bacteria, and is not known to be a pathogen.
- The *bar* gene is composed of the same basic nucleic acids as found in any DNA from known food constituents consumed as part of human or animal diets.
- The acetyltransferase protein family is ubiquitous in nature, including in food and feed products and their function is well-known. No adverse health effects have been related to these compounds.
- The PAT protein has no sequence homology with known allergens or toxins. As expected, it has only a high structural similarity with non-toxic and non-allergenic proteins of the same functional family, in particular with the PAT protein encoded by the *pat* gene.
- The PAT protein has no glycosylation sites.
- The PAT protein is not stable in an acidic environment.
- The PAT protein is rapidly degraded and denatured in human simulated gastric and intestinal fluids.

The production source of the protein was also investigated:

The source of the introduced gene is one of the key variables to consider when performing the safety assessment. Therefore, it is particularly relevant to determine if the donor organism exhibits characteristics of pathogenicity, toxicity or allergenicity.

The *bar* gene was isolated from genomic DNA of *Streptomyces hygroscopicus*, strain ATCC21705 (Murakami 1986 #A50613). The species, *Streptomyces hygroscopicus* (Waksman #C017456), belongs to the grey spore color series and is also known by the subjective synonyms; *Streptomyces endus, Streptomyces violaceusniger (Actinomyces violaceoniger), Streptomyces melanosporofaciens* and *Streptomyces sparsogenes* (Locci 1989 #A54637). An important taxonomic test for the "hygroscopic" species of *Streptomyces* is spore surface morphology determined by electron microscopy (Kutzner 1981 #C017457).

The allergenicity was further studied by comparing protein homology:

2.1 Overall homology

Following the FAO/WHO recommendations, a search for broad homology of the PAT protein to known allergens and toxins was conducted.

The total amino acid sequence of the PAT protein was compared to that of known toxins and allergens listed in 7 large public databases (**Appendix 1**). The algorithm used for the homology comparison was BLASTP and the scoring matrix BLOSUM62. The criterion indicating potential toxicity or allergenicity was a 35 % identity, on a window of 80 amino acids, with a toxin or an allergen.

Based on these *in silico* results, no evidence for any similarity to known toxic or allergenic proteins was found. As expected, the PAT protein only had high structural similarity with other non-toxic and non-allergenic acetyltransferase proteins.

2.2 Potential epitope homology

In addition to the overall homology comparison described above, the FAO/WHO food allergy testing strategy (FAO/WHO 2001 #C023858) recommends conducting a search for epitope homology between the novel protein and known allergens. The purpose of this search is to identify the presence of any short sequence of amino acids that might represent an isolated shared allergenic epitope, which may not be detected by the overall homology analysis.

Although the distinction between allergenic and non-allergenic epitopes is still unclear, a search for any 6 or more contiguous amino acids identical to any segment of any known allergen (food, inhalant or contact allergen) has been recommended by the FAO/WHO. However, this conservative indexing overestimates the number of potentially allergenic proteins. Therefore, it is reasonable to assume that only matches of 8 contiguous and identical amino acids, as recommended previously by the assessment guidelines, have some relevance (WHO/FAO 2000 #C023835). This assumption is based on the fact that the minimum peptide length for a T-cell binding epitope is 8 amino acids (Rothbard #C023827; Metcalfe #C023786). In addition, experimental data (Hileman 2002 #C035251) validate that huge numbers of non-allergens have matching sequences of 7 with known allergens, hence any such event can not be interpreted as indication of an allergenic potential.

It should be noted that sequence-based comparisons as described above are only indicators of potential linear epitopes. The situation is even more complex and less well defined for conformational epitopes. The stability of food allergens to high temperature processing argues for importance of linear, continuous epitopes in assessing potential allergenicity (WHO/FAO 2000 # CO83235) as the linear epitopes are more likely to maintain their structure than more complex epitopes following heat treatment.

The possible effects of glycosylation were also investigated and turn out negative:

Many protein allergens are glycosylated, raising the possibility that the glycosyl groups may contribute to their allergenicity. (Jenkins 1996 #C023834). This is potentially relevant when considering the allergenicity of novel proteins for which glycosylation patterns may differ substantially from their native counterparts.

The best-studied mode of glycosylation is the formation of an N-glycosidic linkage to asparagine in the polypeptide chain. A necessary (but not sufficient) criterion for protein N-glycosylation is the presence of the sequence Asparagine-Xaa-Serine/Threonine (where Xaa represents any amino acid except Proline) in the polypeptide sequence. Although rare, the sequence motif Asparagine-Xaa-Cysteine can also be an acceptor site.

The *in silico* approach enabled the search of potential N-glycosylation sites present in the PAT protein (**Appendix 2**). The results showed that such sites of potential post-translational glycosylations were not found on the PAT protein.

This finding reinforces the fact that the PAT protein does not have glycosylation sites, a characteristic associated with known food allergenic proteins.

The effect of heating on epitope recognition was also studied and it turned out that the antibodies recognised the same epitopes:

5 - HEAT STABILITY

When treated at temperatures up to 90°C for 10 minutes, the PAT protein (encoded by *bar* gene) remains detectable by SDS-PAGE (**Appendix 3**).

By contrast, enzymatic activity of the PAT protein is inhibited at temperatures around 40-45°C, for 15 minutes, and complete thermoinactivation occurs after 10 minutes at 60°C or higher temperatures (Wehrmann 1996 #A57959).

These results show that the immuno-reactivity is still detectable even if the PAT protein loses its enzymatic activity.

In addition, the same recognition of the heat-treated and the native proteins by anti-PAT antibodies indicates that the conformational changes associated with denaturation do not affect the epitope accessibility. This means that the epitope homology search, which showed no similarities with known allergenic epitopes (section III.2), has a major weight of evidence in this safety assessment.

After studying the digestion of the protein conclusions on the allergenicity were positive:

Lack of allergenic potential

- The PAT protein has no amino acid sequence similarity to known allergens. As expected, the PAT protein only has a high structural similarity with other acetyltransferase proteins.
- The PAT protein does not possess potential glycosylation sites, which are often found on allergens.
- The PAT protein is rapidly denatured in an acidic environment. It is rapidly and completely degraded in human simulated gastric and intestinal fluids (between a few seconds and 5 minutes).

The conclusions were published in 2005:

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Toxicology and Pharmacology 41 (2005) 134–149
ELSEVIER
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants

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In conclusion, based on the data available, there seems no real trait for allergenicity with the proposed product.
The PAT protein in it self has no specific properties associated with known allergens.
Some concerns have to be raised:

- since allergy to a specific food is only present in a small number of individuals larger experience will be needed to rule out the possibility to become an allergen.
- not all allergens behave in the same way, as already stated for apple allergen, where very labile allergen (Mal d1) can induce oral allergy syndrome after direct contact with the mucosa of the mouth.
Based on the available data and on a search of the literature, I cannot find evidence that the protein studied has been reported as an allergen hitherto.

Comment 2

No comment, information satisfactory

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

No comment, information satisfactory

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Data seem to be similar to the near-isogenic strain and overall data matches average cotton nutrient composition (Cherry et al., 1978).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

No significant differences shown between LLcotton25 and near-isogenic strain.

D.7.5 Product specification

Comments/Questions of the expert(s)

No comment and information is satisfactory.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

No comment and information is satisfactory.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

No comment and information is satisfactory.

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)

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

No comment

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

As the use of 10% cottonseed meal from LLcotton25 in the diet for broilers was without detrimental effects, we can assume that LLcotton25 is harmless when used within the normal range of incorporation in diets for animal production. In high producing dairy cows a maximum inclusion rate of 15% whole cottonseed is recommended. As approximately 41 % of dehulled cottonseed is represented in cottonseed meal, the results from the broiler study may suggest a safe use of LLcotton25 in other animal species.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

Technical dossier, Part I, P.57: Is it possible to give a detailed list of the anti-nutrients?
P.59, Table 14: It would be interesting to mention the references used to give the range of cotton seed components.
P.60, Table 14: Why is there no reference range for the different classes of fatty acids? How can it be explained that same fatty acid concentrations are lower in the analysed material in comparison with the reference range, while crude fat is within the range?
Why is only vitamin E given in Table 14?
Table 17: does it mean that the use of transgenic cottonseed in animal diets requires more phosphorus, or more phytase?

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

Comments/Questions of the expert(s)

No comment

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

Comments/Questions of the expert(s)

No comment

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)

Page 90, b: Any place receiving sufficient moisture, ports, transit routes, mill surroundings can host volunteer or feral *G.hirsutum* plants. Even more, cotton seedling doesn't need soft soil to root: if seed is spilled down a truck the basis will rotten and become compost, which can constitute a good medium for the germination of few seed of the top of the heap. The only requisite for the plant growth is having enough heat units to reach a growth stage. Cotton growth can occur in southern Europe, South of 42°N. Heat units are calculated according the basic formula:

$$\frac{\text{Daily High} + \text{Daily low}}{2} - 15°C \text{ (developmental threshold)} = \text{Degree Days (DD)}$$

In order to reach a complete cycle, cotton needs about 1300 (°C) DD. So, it is easy to determine in what regions cotton presents a risk of growing out of control.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

At this stage, LLCotton 25 has no selective advantage while in the wild. Exception made for feral cottons along roadsides weeded with the specific glufosinate herbicide.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Gene Flow: Threshold distances measured in USA must be carefully adapted in other regions or continent. Specific tests should be carried out in Europe where cotton growth is possible.
Does the applicant have data from Southern Europe on that respect?

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

No comment

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

Comments/Questions of the expert(s)

No comment

D.9.6 Effects on human health

Comments/Questions of the expert(s)

No comment

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1)

No comment

Comment 2

Gossypol, a typical anti-nutritive factor in native cotton seed, does not seem to result in extra disorders in the case of LLcotton25.
It may be interesting to have a correct explanation for the higher zinc content in LLcotton25 (P.66). However, the zinc content is far below the maximum tolerable level for domestic animals.
Table 20: is the content of crude protein, crude fat and zinc in LLcotton25 significantly different from Coker312?
Table 21 and 22: is the content of crude protein in LLcotton25 significantly different from Coker312? May I assume that differences correspond with $P \leq 0.05$?

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

No comment

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

Comments/Questions of the expert(s)

No comment

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

No comment

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

No comment

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

The issue should be rather presented as follows: it is likely that cotton would establish a feral population but it is less likely that it would be in the vicinity of a commercial cotton field and even less likely that out-crossing would significantly alter overall genetic purity of a commercial field.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

In that case, in EU, who will provide general monitoring on GM cotton and derived substances?

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

Comments/Questions of the expert(s)

At this stage of technical and scientific knowledge and although being aware that the most relevant information comes from studies made in another continent, there is no major risk to introduce cotton seeds and by-products in EU. Nevertheless monitoring of eventual feral populations in harbours, transit road-sides and vicinity of processing plants should be necessary (once a year).

- In EU, are public and private surveillance organisations used in dealing with cotton for environmental impact assessment in cotton (plant identification …)?
- May we have more details about the selected network for information and analysis?
- How reliable “passive surveillance” through voluntary briefing can be?

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

What are the practical arrangements in order to cross-check private sector surveillance reports?

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