Biosafety Advisory Council

12-03-2007

Secretariat

O./ref.: WIV-ISP/BAC/2007_SC_460
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Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/UK/2004/08** of KWS SAAT AG / Monsanto under Regulation (EC) No. 1829/2003 (version 2)

Context

The application EFSA/GMO/UK/2004/08 was submitted by KWS SAAT AG / Monsanto in November 2004 for the marketing of food or feed products produced from or containing ingredients produced from of the glyphosate-tolerant genetically modified sugar beet H7-1 under Regulation (EC) No. 1829/2003[1]. It has been officially acknowledged by EFSA on 20 May 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 5 December 2006 (The EFSA Journal, 2006, 431, 1-18)[2]

On 20 December 2006 the opinion of EFSA was forwarded to the Belgian experts and they were given access to the additional data received from the applicant on request of EFSA. 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

Most of the comments of the experts address scientific questions about discrepancies or unanswered issues in the dossier that could require an improvement of the procedure. This 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.

As the application doesn't imply a cultivation of the plant in EU, a full environmental assessment is not required in EFSA procedure and was not achieved.
According to the Belgian experts, no major risks were identified neither concerning the molecular characterisation, nor the food/feed safety aspects nor the environmental risk and monitoring plan.
There were some concerns expressed about the way some evidences have been gathered:

1) Toxicology tests were achieved on dry pulp but not on raw sugar beet.
2) As in many other applications, tests of allergenicity are based on bioinformatic procedures that are accepted by EFSA. These procedures cover only a part of the allergenicity assessment.

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 GMO panel of EFSA that placing on the market of dried products produced from sugar beet H7-1 are unlikely to have any adverse effect on human and animal health or the environment ;

[2] see: http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/741.html

b) Is of the opinion that further tests should address the toxicity of undried material if those should be placed on the market.

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.

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/UK/2004/08 (ref: BAC_2006_PT_330)

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

1. Molecular characterisation

The breeding lines that were used for genetic and stability studies are explained nowhere: are they homozygous or hemizygous (in certain cases it could be deduced from the text, but this should be clearly in the descriptions). On page 46 of the Technical dossier lines 6401VH and 3 progeny lines resulting from self pollination or crosses (64801H, 74922H and 83002S) are mentioned (with reference to Kraus 2000 for details). Table 10 mentions also other lines. In annex II page 7 two other lines are mentioned (9002RR2 as homozygous and hybrid 5046MK).

2. Food/feed safety assessment

2.1 Toxicological assessment of the whole GM food/feed

About the single functional protein expressed from the DNA inserted in the genetically modified sugar beet H7-1, the applicant states that CP4 EPSPS rapidly loses activity at low pH as would be encountered in a mammalian digestive tract". No evidence is provided of the possible reactivation of the C4 EPSPS in the intestine.

The 90 days sub-chronic toxicity study was performed in rats by using pulp. But was this test conducted by using wet or dried pulp? If indeed dried pulp was used, knowing that dried pulp does no longer contain the CP4 EPSPS protein, wouldn't it be more interesting to perform a chronical study in which the CP4 EPSPS protein is present in the animal diet?

2.2. Nutritional assessment of GM food/feed

Why are the data on the rat and the sheep feeding studies not shown? Only a summary is given even in the technical dossier.

2.3 Allergenic assessment of GM food/feed

Based on the current knowledge, it seems unlikely that H7-1 be involved in food allergy. This conclusion reflects the current knowledge on the topic, and should be reconsidered and updated according to new data. For example, if EPSPS proteins or alike proteins appear to be described as allergens, or if sugar beet is used for another human finality than sugar.

The applicant refers to Silvanovich and Lee (2003) to state that the level of CP4 EPSPS in refined sugar from H7-1 sugar beet is below 0.002 ppm. The reviewer did not find such result in the aforementioned reference.

The fact that CP4 EPSPS is labile to digestion does not exclude that it can be an allergen. Some allergens are known to be heat-labile and to be degraded readily by the digestive system (for review, see Metcalfe 2005). One known example is Mal d 1, the major allergen of apple (Jensen-Jarolim et al. 1999). In addition, it is current knowledge that immediate allergic reactions may occur locally at the level of the mouth or the throat even before the allergen has reached the stomach. Therefore, not only proteins resistant to digestion can be considered as potential allergens.

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/UK/2004/08 (ref: BAC_2006_PT_330)

Bioveiligheidsraad
Conseil de Biosécurité

9 March 2006

Secretariaat
Secrétariat

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Expertise report for the EFSA dossier GMO/UK/2004/08 - 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: Philippe Baret (UCL)
Experts: Pascal Cadot (KUL), Eddy Decuypere (KUL), Godelieve Gheysen (UGent), Peter Smet (Consulter), Wim Stevens (UA)
Domains of expertise of experts involved: Genome analysis, epigenetics, genetic engineering, immunology, alimentary allergology, animal nutrition, toxicology in vivo, endocrinology, physiology, sugar beet, GMO traceability, labelling of food/feed, consumer information.
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2004/08** concerns a notification of the company **KWS SAAT AG / Monsanto** for the marketing of the genetically modified **sugar beet H7-1** for food and feed applications under Regulation (EC) 1829/2003.
The notification has been officially acknowledged by EFSA on 20 May 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) allergenicity, 3) toxicity and/or 4) food and feed aspects. Its was expected that the expert should evaluate if the information provided in the notification is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94).

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

What can be told about the consumption of herbicides on a field with non-GM crops relative to a field with GM (but otherwise identical) crops. Do farmers use more herbicide per surface unit since their GM plant is insensitive? Are there any results available (obtained by an independent organization)?

Comment 2

General comment on the Technical Dossier:
Several references are made to texts that are not available (as far as I know), not that the data in these texts are always really necessary to evaluate the dossier, but what is the purpose of referring to them if they cannot be consulted e.g. Kraus 2000, 2003; Loock 1999, 2000)?
For comments specifically on the Technical Dossier, see below.
Comments on the summary:

- Mistake in text page 10: sugar beet plants are highly **in**compatible (the text says highly compatible)
- Page 18: 'the protein is not detectable using a PCR base method" : is this the immuno-quantitative PCR for protein detection (Gofflot et al., 2004; patent WO 0131056 Zorzi et al 2001) or is it a mistake and should it be PCR for DNA detection/ELISA for protein detection?

Comments on annexes:
Annex II and III: use . instead of , for indicating decimals e.g. table page 9 and following (also in text).
Comments on PCR-detection method:

- Page 6 : of course the lack of homology of beet glutamine synthetase to other plant sequences is due to the lack of sequences from related plants in the database. It might be a better idea to compare the GS sequence with other known GS sequences from other food plants that could also be present in the same food mixture (corn, potato, tomato,…). Most likely the homology will indeed be too low to amplify the sequence with the sugar beet primers (as shown in the table on page 8).
- The PCR detection method for the presence of transgenic H7-1 sugar beet was established on sugar beet seeds. Since part of the transgenic material will constitute of pulp, effect of this matrix should be tested (not done in the analysis of matrix effects page 15).

Comment 3

H7-1 sugar beet is resistant or tolerant to glyphosate, the active component in round-up.
The phosphonomethyl-glycine blocks the activity of EPSPS which is a key enzyme in the shikimic pathway leading to the formation of aromatic amino acids (tyrosine, phenylalanine and tryptophane) in plants, bacteria and fungi but not in animals.
Why then in some text books or dictionaries is a low toxicity in animals mentioned ? Has this enzyme other known functions ? Or is the term "low toxicity" misused ?

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

Comments/Questions of the expert(s)

No questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Table 4: ori-V is called a 'vegetative' origin of DNA replication, ori-322 is called a 'plasmid' origin of DNA replication, is there any difference in type of origin for these two?

Comment 2

What exactly is the difference between the plant EPSPS and the EPSPS from Agrobacterium CP4 so that glyphosate does not block the latter but does so with the plant EPSPS ? This I could not find in the document.
As most bacterial EPSPS also are not tolerant on glyphosate (p 26 in document), I wonder if fungal EPSPS are ?
The use of sugar beet H7-1 resistant to round-up will promote its use; what will be the effect of round-up on soil life (bacteria, fungi) ?

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)

Only the CP4-EPSPS gene in a single copy is introduced in H7-1 sugar beet; no questions.

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

Comments/Questions of the expert(s)

Comment 1

The southern analysis was done quite thoroughly and the results described in sufficient detail.

The breeding lines that were used for genetic and stability studies are explained nowhere: are they homozygous or hemizygous (in certain cases it could be deduced from the text, but according to me, this should be clearly in the descriptions). On page 46 of the Technical dossier lines 6401VH and 3 progeny lines resulting from self pollination or crosses (64801H, 74922H and 83002S) are mentioned (with reference to Kraus 2000 for details). Table 10 mentions also other lines. In annex II page 7 two other lines are mentioned (9002RR2 as homozygous and hybrid 5046MK).

Comment 2

No questions

D.3. Information on the expression of the insert

Comments/Questions of the expert(s)

Comment 1

Figure 15: please give some more details in the legend: what is the unit being used?

Table 6: although at first sight it might seem strange to pool samples for an expression analysis (averaging the variation), of course this is the way the samples will be processed during commercial production, and therefore it is the most appropriate way of analysis.

Comment 2

All elements of the insert are intact; no backbone sequence of the plasmid used is found back.
Expression of CP4-EPSPS in the total plant, leaf and root.
Very little or no chance of transcription in parts that overlap with the 5' and 3' junctions between DNA insert and sugar beet DNA.

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

Comments/Questions of the expert(s)

Comment 1

In some cases germination efficiency appears lower in the transgenic line (most likely due to environmental influence), and nevertheless, if it would be lower, there the transgenic line would be less 'invasive' than the parental line.

Comment 2

No questions

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

Comments/Questions of the expert(s)

No questions

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

Comments/Questions of the expert(s)

No questions

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

Introduction of the CP4EPSPS protein in sugar beet

The product was studied using comparison of the amino acid sequence of the product with known allergens.

In the safety assessment of proteins introduced into genetically modified crops, possible animal and human health effects are addressed in a multifaceted approach which involves addressing the history of safe use, directly assessing the oral acute toxicity of the introduced protein, assessing the sensitivity to rapid digestion and comparing the amino acid sequence of the introduced protein with proteins associated with toxicity, allergenicity or other health effects. A biologically relevant sequence similarity to a known allergen or gliadin (*i.e.* a sequence derived from a common ancestor gene and/or containing a potential allergenic epitope) may indicate that additional immunological assessments should be performed.

A database of protein sequences associated with allergy and coeliac disease was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt) and from current literature. The keyword "allergen" was used to retrieve allergen sequences from the public domain databases. Additional unique allergens found in only current literature were appended creating a database containing 567 unique protein sequences. The amino acid sequence of the CP4 EPSPS protein was compared to these sequences using the sequence alignment tool FASTA. The test protein sequence, CP4 EPSPS, shared no structurally significant sequence similarity to sequences within the allergen database.

In addition, the amino acid sequence of the CP4 EPSPS protein was compared to the allergen database using an algorithm (IDENTITYSEARCH) that scans for a window of 8 linearly contiguous identical amino acids. The CP4 EPSPS protein sequence does not share 8 linearly contiguous amino acid identities to any sequence in the allergen database. These data establish that the CP4 EPSPS protein does not share any immunologically significant sequence similarity to proteins associated with allergy or coeliac disease.

Similar results were obtained by McCoy 2003.

6.0 Conclusions

Analyses of putative polypeptides encoded by DNA spanning the 5' and 3' junctions of the insert T-DNA in Roundup Ready sugar beet event H7-1 into the genomic DNA were performed using bioinformatic tools. Results of the FASTA sequence alignments demonstrated a lack of structurally relevant similarity between any known toxic, pharmacologically active, or allergenic proteins and the nine putative polypeptides. Moreover, results from the epitope match algorithm (ALLERGENSEARCH) demonstrated the lack of potential immunologically relevant sequences between any of the putative polypeptides and the AD4 database. The results of these bioinformatic analyses demonstrate that even in the highly unlikely event that any of the junction polypeptides were translated, they would not share a sufficient degree of sequence similarity or identity to indicate that they would be potentially toxic, allergenic, or have other health implications.

5.0 Results and Discussion

5.1 *Allergen and gliadin database (UPDATE2).* A combination of keyword searches using the STRINGSEARCH function of GCG and Internet searches against publicly available genetic and literature databases was performed to create a database (UPDATE2) containing 567 unique allergen and gliadin protein sequences (see Section 4.1 for a full description). The entire list of proteins in UPDATE2 is shown in Appendix 2. This database represents a comprehensive list of allergens and gliadins.

5.2 *CP4 EPSPS protein sequence is not similar to allergens and gliadins.* A similarity search of the allergen and gliadin database (UPDATE2) revealed no sequences with significant similarity to the CP4 EPSPS protein. The FASTA sequence alignment tool was used to identify overall similarity (structural similarity) as well as identify local (immunologically relevant) similarity to proteins within the allergen and gliadin database. The criteria for a immunologically relevant similarity was defined as an exact match to 8 or more linearly contiguous amino acids (Metcalfe et al., 1996).

The best sequence similarity generated to the CP4 EPSPS protein sequence and the database was an 82 amino acid overlap to the *Dermatophagoides farniae* (mite) protein, Der f 2. This protein shares 30.5% identical residues and 62.2% similar residues within the overlap. The level of similarity is not biologically relevant (Doolittle, 1990) and does not indicate structural homology. Further, a local similarity of 8 or more amino acids was not observed between the CP4 EPSPS protein sequence and sequences within the UPDATE2 database. These data demonstrate the lack of both structurally and immunologically relevant

sequence similarity between the CP4 EPSPS protein sequence and proteins within the allergen and gliadin database.

The complete FASTA output, including sequence alignments, for the CP4 EPSPS search is shown in Attachment 3. The negative control sequence (shuffled CP4 EPSPS protein sequence) also revealed no significant similarities. It was observed that the CP4 EPSPS protein was no more likely to share sequence similarity with allergens and gliadins than the randomized (amino acid shuffled) CP4 EPSPS protein sequence.

5.3 *CP4 EPSPS protein sequence does not contain immunologically significant allergen epitopes.* An algorithm was developed to compare the CP4 EPSPS protein to the allergen database. This program was used to scan for a match of 8 (or more) linearly contiguous identical amino acids between a test sequence and any protein in the allergen database. This algorithm may be more sensitive than the FASTA sequence alignment tool. The window size of 8 amino acids has been previously justified in the literature (Metcalfe et al., 1996).

The complete IDENTITYSEARCH output file for the CP4 EPSPS protein is shown in Appendix 4. No sequences in the allergen database (UPDATE2) were identified to contain a match of 8 or more linear amino acids to the CP4 EPSPS protein.

6.0 Conclusions

The retrieval strategy employed in this study was effective and allowed the assembly of a comprehensive allergen and gliadin database. The FASTA sequence alignment tool was used to compare the CP4 EPSPS protein sequence to sequences contained in this database. Results of the FASTA sequence alignments demonstrated the lack of structurally relevant similarity between the CP4 EPSPS protein and any known allergen or gliadin. Additionally, a computer algorithm (IDENTITYSEARCH) was developed to determine if the CP4 EPSPS protein sequence shared a match of 8 or more linearly contiguous amino acid identities to any sequence within the allergen and gliadin database. Results from this linear scanning algorithm demonstrated the lack of potential immunologically relevant sequences in the CP4 EPSPS protein sequence. Combined, these data demonstrate that the CP4 EPSPS protein is not similar to proteins associated with allergens or coeliac disease.

Some similarity was found with the *Dermatophagoides phagoides* allergen 2 but the amino acid identity was not significant (Rice 2001). Nevertheless since a 30 % homology was found caution has to be pronounced and it would be an advantage to be able to compare the 3D structure of the proteins and to have some blotting experiments with the protein and anti-PROfar 2 IgE.

Bioinformatic analyses were performed on the CP4 EPSPS protein to assess the potential similarity to allergens or other pharmacologically active proteins. A summary of the best similarity from each analysis is shown in Table 1. Supporting dataset output files for each analysis are provided in Appendices 2-4.

Table 1. Best similarities observed to the CP4 EPSPS protein sequence from FASTA searches against ALLERGEN3 and the ALLPEPTIDES protein databases.

Database	Appendix	# Hits	Accession #	Description	E score	% Identity	aa Overlap
ALLERGEN3[a]	2	1	S70378	Der f 2[c]	0.27	30.5	82
ALLPEPTIDES[b]	4	3	Q59975	Phosphoshikimate carboxyvinyl transferase	3×10^{-62}	46.5	443

[a] ALLERGEN3 database corresponds to the allergen and gliadin protein database (Appendix 1).
[b] The ALLPEPTIDES protein database was comprised of SwissProt release 38+, TrEMBL and GenPept release 116.
[c] Der f 2 is a group 2 house dust mite allergen from *Dermatophagoides farinae*.

5.1 *Allergenic assessment of the CP4 EPSPS protein*

Potential structural similarities shared between the CP4 EPSPS protein and proteins in the allergen and gliadin database were evaluated using the FASTA sequence alignment tool. Identified proteins were ranked according to their degree of similarity (Appendix 2). The best similarity observed (Table 1) was to the *Dermatophagoides farniae* (mite) allergen, Der f 2 (Accession No. S70378). In this alignment, the overlap of 82 aa contained seven gaps and was relatively short compared to the length (455 aa) of the CP4 EPSPS protein. Consequently, homology between the CP4 EPSPS protein and Der f II allergen can not be inferred. Frequently, alignments comprised of ≥ 50% identities in short overlaps (20-40 aa) occur by chance and do not indicate homology (Pearson, 1996). Furthermore, recognition of the Der f 2 protein by IgE antibodies depends strongly on the conformation of the protein. Truncation of N- or C-terminal short sequences, destruction of the

In conclusion there is little evidence that the transformed sugar beet will induce allergy more often than the natural variant, to which pollen allergic reactions seldom develop.

Some attention has to be paid to the possible homology to *D. farinae* 2 antigen.

<u>*Comment 2*</u>

No differences in the proximate analysis (dry matter, fibre, ash, fat) or in quality analysis.
However, tyrosine is always statistically lower in leafs of H7-1 beets (and since tyrosine is one of the aromatic amino acids, hence a product of the shikimic pathway which is a target of the enzyme blocked by glyphosate) this could be drawn more to the attention.
However, tyrosine was higher in the roots of H7-1 sugar beets, and this strengthen again the conclusion that some differences found are not considered to be biologically meaningful.
Moreover, mean values of the statistically different amino acids between control and H7-1 sugar beets are in the range of the control values for the conventional control varieties as well as for the near-isogenic controls.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

No questions

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

No questions

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

No questions

D.7.5 Product specification

Comments/Questions of the expert(s)

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

No questions

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Human exposure to CP-4-EPSPS will be negligible except perhaps in regional specialities (see p 99); therefore the animal trials are meaningful.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Bio informatics analysis of the DNA sequences flanking the 5' and 3' junctions of H7-1 sugar beet insert showed no biologically relevant structural similarities to allergens, toxins or pharmacologically active peptides, for any of the putative polypeptides; moreover there is little or no chance to have transcripts except as for the CP-4-EPSPS transcript (see D3).
On this basis, there is little or no theoretical chance for toxicity or allergenicity.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Pg 98 of the technical dossier : "CP4 EPSPS rapidly loses activity at low pH as would be encountered in a mammalian digestive tract"

Pg 23 of Padgette 1993 : "pH dependence of CP4 EPSPS activity and stability : These results indicate that the majority of enzymatic activity is not irreversibly lost at either low or high pH range"

Pg 99 of the technical dossier : "If the CP4 EPSPS protein was not completely digested by the gastric system, it would be rapidly degraded in the intestine"

Suppose the CP4 EPSPS reaches (undigested) the intestine. With the pH being between 7 and 8, the enzyme can be reactivated. Which are the possible consequences of this?

Comment 2

Pg 98 of the technical dossier : "The CP4 EPSPS protein from *E. coli* is a suitable substitute for sugar beet-produced CP4 EPSPS."

Under section D.3.a.i., the characterization of the CP4 EPSPS protein is described.

As far as I can ascertain, no scientific proof is given that the CP4 EPSPS protein in sugar beet is identical to the CP4 EPSPS protein produced by *E. coli*. What is the reliability of the acute toxicity test, performed by the use of the protein obtained from *E. coli?*

Comment 3

What if raw (unprocessed) sugar beet is used as feed (e.g. non-professional cattle-breeders)?
These animals are exposed directly to the protein. In these cases, what is the magnitude of the exposure concentrations? Are these covered by the range of test concentrations used in the acute toxicity test?

Comment 4

Safety assessment of the nearly expressed protein was based on:

- protein specificity
- no homology with known protein toxins
- very quickly digested in vitro (stomach and intestinal milieu), much quicker than the normal transit time of feed in these compartments: therefore, very little chance that the intestine would be exposed to possible feed allergens; if any present
- no acute toxicity

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Not applicable

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

No questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

The 90 days study was performed by using pulp.
In section 7.6.2, it was mentioned that dried pulp does no longer contain the CP4 EPSPS protein.
Was this test conducted by using wet or dried pulp? As far as I can ascertain (Kirkpatrick 2003), dried pulp was used.
If indeed dried pulp was used, wouldn't it be more interesting to perform a chronical study in which the CP4 EPSPS protein is present in the animal diet?

Comment 2

Why are the data on the rat and the sheep (D7.10.2) feeding studies not shown? Only a summary is given even in the technical dossier. I am not supposed to evaluate this part because it is not my expertise, but I don't believe that these aspects can be evaluated without the real data shown.

Comment 3

No questions

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

In section 7.9.1 (i).
The reviewer agrees when the applicant states that the CP4 EPSPS gene was obtained from a source not known to be allergenic.
The reviewer agrees when the applicant states that the amino-acid sequence of CP4 EPSPS does not match the sequence of any known allergen or that of protein involved in coeliac disease.

In section 7.9.1 (ii).
The fact that CP4 EPSPS is labile to digestion does not exclude that it can be an allergen. Some allergens are known to be heat-labile and to be degraded readily by the digestive system (for review, see Metcalfe 2005). One known example is Mal d 1, the major allergen of apple (Jensen-Jarolim et al. 1999). In addition, it is current knowledge that immediate allergic reactions may occur locally at the level of the mouth or the throat even before the allergen has reached the stomach. Therefore, not only proteins resistant to digestion can be considered as potential allergens.
Another statement by the applicant does not seem relevant, when it is said that allergens are present as major components in the specific food (see Metcalfe 2005). It is current knowledge that minute amounts of allergen are sufficient to elicit an allergic reaction. The amount of transferred protein cannot strictly be used as an argument to preclude its allergenicity.

However, it is true that, for the present application, the final product consumed by humans is refined sugar, in which little beet protein remains after processing (maximum 1 ppm, according to Potter et al 1990). The level of a specific protein is expected to be even lower. For information, the lowest level of protein ever described to have elicited an allergic reaction is around 100 µg (Monneret-Vautrin & Kanny 2004). This corresponds to 100 g of food containing 1 ppm of offending proteins. In those conditions, and given the negative allergenic history of sugar beet and the absence of known allergy to CP4 EPSPS, it seems unlikely that H7-1 be involved in food allergy.
This conclusion reflects the current knowledge on the topic, and should be reconsidered and updated according to new data. For example, if EPSPS proteins or alike proteins appear to be described as allergens, or if sugar beet is used for another human finality than sugar.

Remark: The applicant refers to Silvanovich and Lee (2003) to state that the level of CP4 EPSPS in refined sugar from H7-1 sugar beet is below 0.002 ppm. The reviewer did not find such result in the aforementioned reference. In addition, in the summary, section 7.9.1, it is said that the CP4 EPSPS protein was not detectable in sugar from H7-1 beet, using a PCR method. The reviewer does not understand how a PCR method can be used to measure protein levels.
The applicant is kindly asked to check the validity of those data (reference and measurement).

Sugar beet pollen allergy is a rare affection (Hohenleutner et al 1996, for the most recent reference). One of the main reasons is that sugar beet roots are collected before the plant flowers and emits pollen. However, occupational allergy to sugar beet pollen has already been documented and care should be taken that it does not become more common because of the artificial introduction of a new trait.
For that reason, the applicant should determine whether CP4 EPSPS is present in the pollen of H7-1 sugar beet. Although proteins of the family of CP4 EPSPS have never been described as allergens, contact through the respiratory tract might represent a new way of exposure, with unknown outcome, and a possible way of sensitization not existing previously. Therefore, the level of expression of CP4 EPSPS in pollen is an important issue to be addressed. The same double antibody sandwich ELISA, as described in section D3 (a) (ii) can easily be used to probe a pollen protein extract.

Comment 2

See 7.8

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

No questions

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

Comments/Questions of the expert(s)

No questions

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

Comments/Questions of the expert(s)

No questions

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)

NOT APPLICABLE

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

NOT APPLICABLE

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

NOT APPLICABLE

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

NOT APPLICABLE

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

Comments/Questions of the expert(s)

NOT APPLICABLE

D.9.6 Effects on human health

Comments/Questions of the expert(s)

No effects on in vivo tests with Sprague-Dawley rats based on performance, clinical examination, haematology, serum chemistry and urine analysis.
However, for the latter two no list of parameters measured are given: why ? On what basis ?

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

The same remark for evaluation of nutritional characteristics of sugar beets in sheep trial: why are the data not given, but only the conclusions ?

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

NOT APPLICABLE

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

Comments/Questions of the expert(s)

NOT APPLICABLE

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

NOT APPLICABLE

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

See remark under C
Since the key metabolic pathway in the biosynthesis of the aromatic amino acids, tyrosine phenylalanine and tryptophane occurs in plants, bacteria and fungi, but not in animals, the glyphosate blocks the synthesis of aromatic amino acids by interfering with the shikimic pathway in all of them except in some species where the EPSPS is not sensitive for glyphosate.
Therefore, the generalized use of round-up may not only affect weeds but also micro-organisms and fungi in the soil, hence, soil life, unless persistence in soil is very low, or very low quantities of glyphosate reach the soil when applied in the leafs. These aspects are not at all discussed in the application, and I cannot judge if this is considered in the environmental monitoring plan for the H7-1 sugar beet notification under Directive 2001/18/EC (nr. C/DE/00/8).

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

NOT APPLICABLE

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

NOT APPLICABLE

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

Comments/Questions of the expert(s)

NOT APPLICABLE

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

NOT APPLICABLE

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