Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-UK-2006-34 (maize 3272) from Syngenta under Regulation (EC) No. 1829/2003

4 February 2020
Ref. SC/1510/BAC/2020_0145

Context

Application EFSA-GMO-UK-2006-34 was submitted by Syngenta for the authorisation for the marketing of genetically modified (GM) maize 3272 for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

Maize 3272 contains a single insert consisting of the *amy797E* and the *pmi* cassettes, expressing a thermotolerant alpha-amylase (AMY797E) and a phosphamannose isomerase (PMI) as a selectable marker.

The application was validated by EFSA on 6 July 2007 and a formal three-month consultation period of the Member States was started, lasting until 6 October 2007, 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). Seven experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments and the comments sent to EFSA.

The opinion of the EFSA Scientific Panel on GMOs was published on 20 June 2013 (EFSA Journal 2013;11(6):3252[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. This opinion was inconclusive because of insufficient data provided for the comparative assessment and uncertainties regarding the *de novo* sensitisation potential of the newly expressed protein AMY797E. Following the submission by the applicant of additional data, the EFSA GMO Panel was requested to complement its original opinion. The "Statement Complementing EFSA Scientific Opinion" on maize 3272 was published on 4 November 2019 (EFSA Journal 2019;17(11):5844[3]).

In delivering the present advice the BAC considered in particular the information below:
- The comments formulated by the experts on application EFSA-GMO-UK-2006-34;
- The opinion of EFSA from 2013;
- The complementing statement from 2019.

[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 https://doi.org/10.2903/j.efsa.2013.3252
[3] See https://doi.org/10.2903/j.efsa.2019.5844

Biosafety Advisory Council - Secretariat • Service Biosafety and Biotechnology (SBB)
Sciensano • Rue Juliette Wytsmanstraat 14 • B-1050 Brussels • Belgium
T + 32 2 642 52 93 • bac@sciensano.be • www.bio-council.be

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize 3272 (i.e. during transport and/or processing) into the European environment[4] will lead to environmental harm.

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM maize 3272, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of GM maize 3272, in comparison with its conventional counterpart, does not raise safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed PMI protein in the context of previous applications, and no concerns were identified. Since no new information on allergenicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid.

The thermotolerant alpha-amylase AMY797E has not been evaluated previously by the Biosafety Advisory Council. In the framework of this application, the Council's questions regarding the allergenicity of this newly expressed protein in the context of its use in whole food have not been answered satisfactorily. As a consequence, the Biosafety Advisory Council is unable to determine whether GM maize 3272 is as safe as conventional maize.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of maize 3272-derived food and feed are not expected to differ from those of conventional maize varieties.

4. Monitoring

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

[4] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment is as in the case of a cultivation file is not warranted.

Conclusion

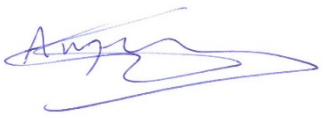
Based on the whole set of data on maize 3272 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion and complementing statement of EFSA, and the answers of the EFSA GMO panel to the questions raised by the Belgian experts, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of maize 3272 is unlikely to pose any threat to the European environment;

2) Agrees with the GMO panel of EFSA that in the context of its proposed uses as DDGS for feed, maize 3272 is unlikely to pose any risk to human and animal health;

3) Considers however that since this application is for both food and feed uses under Regulation (EC) No. 1829/2003, and since there are remaining uncertainties concerning the allergenicity of the whole food derived from the GM plant, it is not possible to draw a final conclusion on the food safety of maize 3272.

In addition the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM maize in the existing allergenicity monitoring systems.



Prof. Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-UK-2006-34 (ref. BAC_2007_PT_585) and comments sent to EFSA (ref. BAC_2007_PT_586)

Bioveiligheidsraad
Conseil de Biosécurité

28/09/2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT_585
Email. : bac@sbb.ihe.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2006/34

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 10 August 2007
Coordinator: Prof. Dirk Reheul
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Patrick du Jardin (FUSAGx), Leo Fiems (ILVO), André Huyghebaert (UGent), Nancy Teryn (UGent) and Jan Van Doorselaere (Katholieke Hogeschool Zuid-West Vlaanderen)
Domains of expertise of experts involved: agronomy, breeding, molecular characterisation, genetic engineering, genome analysis, ecotoxicology, animal and human nutrition, analysis food/feed, allergology, immunology, maize
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2006/34** concerns an application of the company **Syngenta Seeds S.A.S.** for the marketing of the genetically modified **maize event 3272** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 6 July 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).

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1
In some instances I could not consult the papers referenced to in the text (some of the internet links referred to could not be reached and the papers were not available on the CD in the reference folder. E.g. EuropaBio). It would be useful if all papers referenced to would be on the CD

In the technical dossier part I page 8, it is stated that the application also covers the import and processing of Event 3272 *for all potential uses*.
In the next paragraph it is stated that the grain is not intended to be exported as a commodity crop.
Q: What measures are taken to prevent that the grain is exported as a commodity crop? Is the price expected to be lower than that of conventional corn grain?

Extra info per mail (20/09) : Wat de referentie betreft: omdat ik verbaasd was te lezen dat de 5 meest voorkomende vetzuren 90% van de totale lipiden van mais graan uitmaken (EuropaBio, 2003) (Deel I, Appendix 8, vol 1, blz 12) wou ik toch even checken of dit inderdaad gepubliceerd is. (Door berekening die ik gemaakt heb op grond van de analyseresultaten in het dossier omtrent het vetgehalte en deze 5 vetzuren bekomt men 90%; mocht deze waarde gepubliceerd zijn is dit een goede indicator voor de degelijkheid van de analyseresultaten). De link naar deze website is gegeven of blz 15 van (Deel I, Appendix 8, vol 1). Deze link kon ik niet bereiken.

Comment 2
No comment/question

Comment 3
Event 3272 maize has been specially modified for industrial use in fuel ethanol production. In theory, there may be no problems for human and animal safety as this maize is not intended to be used for food and feed. However, the fact that Event 3272 may enter international trade routes necessitates a careful approach of this GM maize. What does Syngenta mean by "a low level" (Technical dossier, p. 8, § 5)? Is it possible to give a maximum level, and what are the consequences for safety when Event 3272 maize is included above this maximum rate?
Moreover, as maize contains a large amount of starch, the alpha-amylase (AMY) introduced into Event 3272 may have an important effect on this component with regard to the utilization in human nutrition and animal feeding.

Comment 4
Event 3272 is a genetically modified (GM) maize that has been developed to serve as the source of alpha-amylase enzyme in the dry-grind ethanol process from maize, replacing the external addition of microbially produced enzyme. Event 3272 maize is intended to be cultivated outside the EU. The grain will be locally used in the dry-grind fuel ethanol process. However it cannot be excluded that the harvest originally intended to be used in the dry-grind fuel ethanol industry could finally enter international trade routes albeit at an extremely low level. By-products of the dry-grind ethanol

process produced from maize are used as feed and are exported to the EU (e.g. Distillers Dried Grains and Solubles).

A small inconsistency to my opinion, on if the product been notified in a third country either previously or simultaneously? Page 4 summary says US and China, on page 26 it is Japan and US.

Comment 5:
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

Additional comment of the coordinator

The dossier does not provide information on 1) how the Event was introgressed from the initially transformed line into other inbred lines 2) witch inbred lines were used to create whatever hybrid 3) with witch genetic material (inbred lines, different hybrids, ….) agronomic, animal and other trials and laboratory experiments were conducted. This is essential information as it has been demonstrated that genes might be differently expressed in different genetic backgrounds.

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

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
Event 3272 is a genetically modified (GM) maize, which expresses two transgenes:
A synthetic *amy797E* gene encoding the thermostable AMY797E alpha-amylase protein and the *pmi* (*manA*) gene from *Escherichia coli,* which encodes the enzyme PMI as a selectable marker.

Comment 3
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1

1. **Southern blot analysis of backbone sequence.** The applicant concludes that no backbone sequence from the recombinant T-DNA plasmid pNOV7013 was inserted in the maize genome, based on the Southern blot analysis presented in figure 12 of CBI-Appendix 1. However, both the positive and negative segregants of the BC4 generation used in this analysis show faint, but discrete, hybridized bands using the full 'backbone probe', which are not commented by the applicant. Such comments are awaited, and additional Southern blots should be performed, using parental, non-transformed lines as additional negative controls. Insertion of backbone sequences in a different locus could produce such bands in so called BC4 + and – segregants (remind they are named in that way based on the PCR testing of plants using primers corresponding to the transgenes only). The Southern blot analysis could also be complemented with PCR TaqMan analysis with primers deduced from the backbone sequence, especially the antibiotic resistance *aadA* gene of safety concern, using appropriate controls.
2. **Potential novel ORFs at the junction regions :** The applicant defines a potentially functional ORF as " a region corresponding to at least fifty aa in length …" (see page 6 of CBI-appendix 4) and uses this criterium in the bioinformatic analysis of the junctions between the inserted T-DNA and the target locus. Peptides with biological functions may be much shorter than 50 aa, hence the bioinformatics analysis should be repeated by analysing all possible ORFs of much shorter size (*e.g.* down to 3 codons).
3. **Regions of homology between the 3'flanking region and maize genomic DNA.** The arguments set by the applicant in CBI-Appendix 4 – 'it is not likely that the maize genomic sequence flanking the 3' region of the event 3272 T-DNA insert could function as a promoter

as the maize genomic sequence is missing core components necessary for promoter function '- are not very convincing. However, the 'weight of evidence' approach of the GM plants raises no safety concern (no unintended phenotypic effects, see section D7.4 on Agronomic traits).

Comment 2
Adequate molecular techniques were used to characterise the transformation event and insertion. Molecular analysis shows that the event contains a single insert and does not retain backbone sequences from the vector.

A small remark: Appendix 1, figure 14, the positive controls, lane 7 and 9, are not visible, but in line with also the weaker bands on the other Southern blots, I assume that this is OK.

No relevant homologies of the flanking plant DNA regions were found (Appendix 4- dated Jan 2006). I just wondered whether as new genomic sequences appear every day in the database (to my knowledge the Zea mays genome has not been fully sequenced?) these searches have been done recently. I guess there is a system that has it monitored?

On P19 of the Technical Dossier it is said that no novel Open Reading Frames (ORFs) were identified that spanned either the 5' or the 3' junctions between Event 3272 T-DNA and *Zea mays* genomic sequences. No fusion proteins are therefore expected. An ORF was defined in the appendix of a region coding at least 50 AA. I checked in other dossiers, and there seems no consensus on to how small an ORF can be. In another file they started from ORF's as small as 100 bp and do all blast analysis and even expression analysis on that. I am not saying that I would like to see this done here, but maybe it is a suggestion for EFSA to give guidelines as to how an ORF is defined and what kind of analysis should be done to prove non functionality of the ORF. Also as small RNA's are getting more in the picture as gene regulators it might be a good idea to set some guidelines. Experts can be asked for their view on this.

Comment 3
Although the characterisation of the T-DNA in the maize genome has been substantially described (eg by means of various Southern-blotting experiments and sequence analysis) I have some additional questions and comments.

Technical dossier Appendix 1

P 6: there is referred two times to figure 15

P 16: the PEPC9 intron is located after the stopcodon of the AMY797E sequence. What is its function? Why is it there?

P18: Blast analysis of the 5' and 3' T-DNA flanking sequences: I do not find information on how these sequences were obtained (inverse-PCR, genome walking, …?).

Can a conclusion be drawn about the chromosomal location of the T-DNA insert (on which chromosome)?
Although some short sequence similarities are found with the 3' sequences (eg chromosomal maize sequence AF391808 and BAC clone c573L14) one can ask the question if these are significant. Why are not similarities found with the 5' sequence since the T-DNA is supposed to insert at one locus.

Why are only short sequence similarities found with the 3' sequences and not sequence identity with the complete 1000 bp flanking sequence? Could it be possible that these short sequences with high sequence identity are due to the presence of genome duplications in the maize genome?
Should the conclusion be drawn that the sequence surrounding the T-DNA insert is not yet available in Genbank?

Conclusion: it would be informative to add more data about the T-DNA flanking regions

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
Validation of the ELISA tests. The dossier presents expression data of both the AMY797E and PMI proteins based on ELISA (CBI Appendix 5). No western blot on the corresponding tissue extracts is shown for the validation of the test, especially for the AMY797E polyclonal antibodies which could show some cross reactivity with endogenous amylases. Such possible cross reactivities are underlined by the applicant (page 27 of the Technical dossier) but not taken into account by the immunoassays. CBI Appendix 10 shows western blots, but on the immunoaffinity purified AMY797E test sample only, regrettably not on plant extracts. Moreover, the maize line used for preparing the test AMY797E-0104 is described in vague terms – 'from transgenic maize grain derived form event 3272' (page 8 of Appendix 10) – and the applicant should be more accurate on this and assure that the test substance corresponds well to the plant materials used in the ELISA tests.

Comment 3
Expression is stable over several generations

Comment 4
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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
No comment/question

Comment 2
The expression of AMY797E and PMI has not changed the agronomic characteristics of conventional maize.

Comment 3
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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 comment/question

Comment 2
The scope of this application does not include authorization for the cultivation of Event 3272 maize in the EU. Gene transfer from Event 3272 maize to other sexually compatible plant species is not possible since there are no maize wild relatives in the EU

Comment 3
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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
In the literature, it is claimed that crude corn oil is/will be extracted from distiller's grain derived from dry mill ethanol factories [Anonymous, 2007]. Although this oil is said to be intended for biodiesel production [Anonymous, 2007], it can not be excluded that it comes into the food chain. Therefore, compositional analysis of such oil derived from genetically modified grains and comparison with that from conventional corn oil may be warranted**.** In the dossier it is stated that the end products of Event

3272 maize grain after the dry-grind ethanol process are not different from those obtained from conventional corn but no data are given.
Q: Are there data on the composition of distiller's grain derived corn oil from Maize 3272 compared to that from distiller's grain from conventional corn?

Low concentrations of AMY797E alpha-amylase were found in the non-transgenic near-isogenic controls (Technical dossier page 31**).**
Q: Were these controls obtained from field trials in the proximity of the genetic modified plants where there could have been gene transfer or is it really "contamination"? If so, why than no contamination with commercially-sourced grain?

Cryptoxanthin levels are expressed as retinol equivalents (which is one way to do this) (Technical Dossier, Appendix A, Vol 1 pp 26) but the literature reference value in microgram/100 g. No convertion factor for mass cryptoxanthin in retinol equivalents is given however . Such a factor is required to see whether the found values are within the literature range as stated. Note that there is a discussion amongst nutritionists what conversion factors of weight to RE would be required for retinoids [de Pee etal., 1998]. Further note that there are more recent papers on cryptoxanthin levels in corn [e.g. de Oliveira et al, 2007; Scott & Elderidge, 2005] and that there is a tendency to express carotenoids in weight/ g [Rodriguez-Amaya, 2003].
Q: What was the convertion factor used to convert weight content of cryptoxanthin into retinol equivalents?

Comment 2
No comment/question

Comment 3
Maize 3272 was compared with relevant non-GM control maize lines, commercial varieties included if possible.
No further questions

Comment 4
The agronomic performance and phenotypic data generated suggest that the genetic modification resulting in Event 3272 did not have any unintended effects

Comment 5
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
AMY was extracted from Event 3272 transgenic maize which is a favourable aspect with regard to

the selection of the material for analysis. However, phosphomannose isomerase (PMI) was produced by recombinant E. coli, because it may be practically impossible to obtain a sufficient amount of plant derived protein. Although the PMI proteins from recombinant E. coli and from Event 3272-derived maize were determined to be substantially equivalent (Technical dossier, p.27), it has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and Schubert, 2004). The fact that forage was also analysed (Technical dossier, p.23) seems not very relevant, as the Event 3272 maize will not be cultivated in the EU and only maize grain will be imported.

Comment 3
Transgenic and corresponding isogenic maize was planted in the US for grain and forage analysis in 2003 and 2004.

Comment 4
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
Key nutrients for analysis of grain and forage were selected with the OECD document as a guide. The analysis includes for grain: proximates, minerals, amino acids, selected fatty acids, vitamins, anti-nutrients and secondary plant metabolites.
Contrary to other dossiers starch is included and calculated by difference. Total dietary fibre was also assessed.
Some statistical differences were found for fibre levels. They are however within the range of natural variations. A similar observation was made, in some cases, for protein and particular amino acid levels. Once again levels are within the range of natural variations.
For forage no consistent statistically differences were found.
I agree with the conclusion that maize 3272 is substantially equivalent to non-GM control maize hybrids.

Comment 3
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.
Has it been tested –fi by means of 2D-gel analysis- if the elevation of alfa-amylase in grain has an effect on the increase or decrease of proteins other then alfa-amylase (fi enzymes involved in starch metabolism)?

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1
No comment

Comment 2
7
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1
No comment

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1
No further comment

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1
Maize 3272, expressing a thermostable enzyme is intended for use in the dry-grind fuel ethanol process. The whole process of starch hydrolysis is summarized, as well as the fermentation, and distillation step.
Final products are ethanol, CO2 and distillers grains and solubles.
Distillers grains and solubles are used in animal feed. A full description of the processing is given.
The applicant concludes that no nutritional changes are to be expected.

I agree with this conclusion.

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1
Neither of the transgenes has been introduced to control other organisms, and the proteins they express (AMY797E and PMI) do not have toxic modes of action,

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
According to Appendix 16 AMY is safe from a toxicological point of view. This has been confirmed by Landry et al. (2003).

Comment 3
Alpha-amylase enzymes from fungal and bacterial sources have a long history of safe use for starch processing in the food processing industry.
Also phosphomannose isomerase proteins have a history of safe use. AMY797E alpha-amylase and PMI are digested rapidly, show a lack of acute toxicity and show no significant homology to known protein toxins. They can therefore be considered non-toxic and unlikely to present a health risk to humans or animals.

Comment 4
Technical dossier P 28: concerning the stability of the PMI protein I would prefere to change the following text:
"… the results of this study showed that the PMI protein is essentially inactivated after incubation at 65°C…" into "… PMI protein activity is strongly reduced (eg 2% residual activity) after incubation at 65°C…"

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1
Not applicable

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1
The dossier includes information about proximates, starch, fibre, a range of minerals and vitamins, amino acids, fatty acids, relevant secondary metabolites and anti-nutrients.
Contrary to previous dossiers I have no questions for further information related to the composition

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
A 90-day safety study in rats and a 49-day poultry feeding study are presented.
I agree with the overall conclusion that maize 3272 is safe for food and feed consumption and no differences in wholesomeness are expected.

Comment 3
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Assessment of allergenicity of the newly expressed proteins

As mentioned by the applicant, AMY797E and PMI are not likely to be allergenic.

Assessment of the allergenicity of the whole GM plant or crop

In section 7.9.2, the allergenicity of the genetically modified maize itself has not been evaluated. The rationale of this section is not to take the new traits into consideration, but to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source.

Although not frequent, food allergy to maize exists and major allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002), and new allergens might be described in the near future (Weichel et al. 2006). The introduction in the plant of AMY797E and of phosphomannose isomerase and the effects thereof might interfere with the expression levels of other maize proteins, including allergens. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified 3272 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize can be carried out.

Comment 2

No questions

Comment 3

The homology searches with known allergens use a 2005 database release in the AMY797 study, and a 2006 release for the PMI study. Authors are requested to use the latest releases for all studies, hence to uptade the AMY797 study.

Comment 4

FAO/WHO (2001) proposes pepsin degradation as a method for the evaluation of allergenicity of genetically modified foods. However, it is not necessarily safe to conclude that a protein that degrades rapidly in simulated gastric fluid is not an allergen. Furthermore, the similarity of amino acids with know allergens was studied as described by FAO/WHO (2001), where a cross-reactivity between the expressed protein and a known allergen hasto be considered when there is:

1) more than 35 % identity in the amino acid sequence of the expressed protein, using a window of 80 amino acids and a suitable gap penalty, or

2) identity of 6 contiguous amino acids. In this dossier, a sequence homology of 8 contiguous amino acids was used, while a six amino acid match is more appropriate and would avoid any false negatives. However, there is no proof that a six or eight amino acid match is predictive in the bioinformatics section. A number of people now recommend not performing the 6-8 amino acid match. There was one region of sequence homology of eight contiguous identical amino acids between PMI and a known allergen, alpha-parvalbumin from *Rana species* CH2001. For the abovementioned reason, it may a false negative.

Simulated gastric fluid (SGF) was used to test the digestion of AMY and PMI proteins The fact that major allergens with high percent allergenicity were not necessarily more resistant to SGF or SIF digestion than allergens with low percent allergenicity renders the use of SGF and SIF

digestibility difficult as a tool to distinguish potential food allergens from nonallergenic proteins (Fu et al., 2002). Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. An additional issue would be the physiological relevance of the test. In reality, protein does not enter the acid environment of the stomach as a pure test solution, but rather as part of a complex food matrix. Within a bolus of food passing through the stomach, it is unlikely that all protein is exposed to the extremes of acid pH, and some protein is likely to survive intact into the lower intestine. One of the properties of food allergens is their thermal stability (Breiteneder and Mills, 2005). PMI is active at ambient temperatures and at 95°C for 30 minutes (Appendix 13). AMY was specially selected for its thermal stability. However, details of the thermal stability are lacking in the dossier in contradiction with PMI.

Comment 5
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
No information dealing with the in vitro organic matter digestibility of Event 3272 maize was find. This is a rapid technique that can provide interesting information. Based on the chemical composition and the vitro organic matter digestibility, the metabolic and net energy can be estimated, yielding extra information for pigs and ruminants. In the poultry feeding study feed efficiency was not different, which may be an indication of a similar digestibility of GM and control maize. It would be interesting to know the effect of AMY in maize grain on starch degradability in the fore stomachs of ruminants. If the starch is more rapidly degraded in the presence of AMY, it may provoke rumen disorders in diets containing a high amount of maize which may be detrimental.

Comment 3
Taking into account the information on the composition and the main use of maize 3272 no nutritional imbalances are expected.

Comment 4:
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1
No questions

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1
Neither of the 2 newly expressed proteins have toxic modes of action, therefore discussions about the interactions between Event 3272 and target organisms are not applicable.

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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 consistent differences in any agronomic characteristics between Event 3272 and control maize were detected.
In addition, no wild relatives of maize are present in Europe. Therefore, maize cannot exchange genes with any other species in the EU (Niebur, 1993).

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
The possibility of gene transfer seems to be very low to negligible because it is not intended to use Event 3272 maize for cultivation.

Comment 3
Gene transfer from Event 3272 maize to other sexually compatible plant species is not possible since there are no maize wild relatives in the EU

Comment 4
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1
Not applicable

Comment 2
This topic is not relevant.

Comment 3
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1
Based on the toxicological studies (Appendix 16, 17 and 19, Annex 9a to 9k) and the poultry feeding study (Annex 21) a safe use of Event 3272 maize can be assumed.

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

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

Comments/Questions of the expert(s)

Comment 1
Not applicable (no cultivation within the scope of this application)

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
No comment = I have read this chapter and corresponding appendices and I agree with the stated information.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
Event 3272 maize is intended to be used in the dry-grind fuel ethanol processing. However it cannot be excluded that the harvest originally intended to be used in this processing could finally enter international trade routes albeit at an extremely low level. It is intended that Event 3272 maize should be used as any other maize in the EU, hence movement and processing have been considered in the development of the monitoring plan.

Comment 3
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1
No comment/question

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1
No comment/question (accepted as 'not applicable' as no specific risk identified by the ERA).

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

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

Comments/Questions of the expert(s)

Comment 1
Although general surveillance of potential, long term, indirect effects poses methodological difficulties, the applicant is too vague on the nature and qualities of the participants of this surveillance, despite the fact that an annual report of the general surveillance activities is announced, complying to the EFSA guidelines. Relying on "an adequate number of people, with relevant experience" (page 4 of Appendix 25) is not precise enough. The applicant should elaborate on this.

Comment 2
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1
ND = Not Done = I read the chapters in the technical dossier and I can agree with the rationale in the provided information but I did not read the corresponding appendices (if present) and therefore I cannot state whether the information provided is correct.

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Bioveiligheidsraad
Conseil de Biosécurité

05/10/2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT_586
Email. : bac@sbb.ihe.be

Application EFSA/GMO/UK/2006/34
Comments submitted on the EFSAnet on mandate of the Biosafety Advisory Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 10 August 2007
Coordinator: Prof. Dirk Reheul
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Patrick du Jardin (FUSAGx), Leo Fiems (ILVO), André Huyghebaert (UGent), Nancy Teryn (UGent) and Jan Van Doorselaere (Katholieke Hogeschool Zuid-West Vlaanderen)
Domains of expertise of experts involved: agronomy, breeding, molecular characterisation, genetic engineering, genome analysis, ecotoxicology, animal and human nutrition, analysis food/feed, allergology, immunology, maize
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2006/34** concerns an application of the company **Syngenta Seeds S.A.S.** for the marketing of the genetically modified **maize event 3272** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 20 June 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).
When needed, comments from the experts have been summarized by the coordinator and for clarity some sentences have been rephrased.
For the full comments of the Belgian experts and the bibliographic references we refer to the document given in annex 2. It displays all the comments as there were transmitted by the experts (ref. BAC_2007_PT_585)

List of comments submitted on the EFSAnet

A. GENERAL INFORMATION

Comments submitted on the EFSAnet

Event 3272 is a genetically modified (GM) maize, which expresses two transgenes:
A synthetic *amy797E* gene encoding the thermostable AMY797E alpha-amylase protein and the *pmi* (*manA*) gene from *Escherichia coli,* which encodes the enzyme PMI as a selectable marker.

Event 3272 is a genetically modified (GM) maize that has been developed to serve as the source of alpha-amylase enzyme in the dry-grind ethanol process from maize, replacing the external addition of microbially produced enzyme. This maize is not intended to
be used for food and feed. However, the fact that Event 3272 may enter international trade
routes necessitates a careful approach of this GM maize. By-products of the dry-grind ethanol process produced from maize are used as feed and are exported to the EU (e.g. Distillers Dried Grains and Solubles). In the technical dossier part I page 8, it is stated that the application also covers the import and processing of Event 3272 for all potential uses.
In the next paragraph it is stated that the grain is not intended to be exported as a commodity crop.
Q: What measures are taken to prevent that the grain is exported as a commodity crop?

What does Syngenta mean by "a low level" (Technical dossier, p. 8, § 5)? Is it possible to give a maximum level, and what are the consequences for safety when Event 3272 maize is included above this maximum rate? Moreover, as maize contains a large amount of starch, the alpha-amylase (AMY) introduced into Event 3272 may have an important effect on this component with regard to the utilization in human nutrition and animal feeding.

In some instances papers referenced to in the text could not be consulted: some of the internet links referred to could not be reached and the papers were not available in the reference folder (E.g. EuropaBio). It would be useful if all papers referenced to would be in the list.

A small inconsistency to my opinion, on if the product been notified in a third country either previously or simultaneously? Page 4 summary says US and China, on page 26 it is Japan and US.

The dossier does not provide information on 1) how the Event was introgressed from the initially transformed line into other inbred lines 2) witch inbred lines were used to create whatever hybrid 3) with witch genetic material (inbred lines, different hybrids, ….) agronomic, animal and other trials and laboratory experiments were conducted. This is essential information as it has been demonstrated that genes might be differently expressed in different genetic backgrounds.

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

Comments submitted on the EFSAnet

None

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments submitted on the EFSAnet

None

D. INFORMATION RELATING TO THE GM PLANT

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

1. Southern blot analysis of backbone sequence.

The applicant concludes that no backbone sequence from the recombinant T-DNA plasmid pNOV7013 was inserted in the maize genome, based on the Southern blot analysis presented in figure 12 of CBI-Appendix 1. However, both the positive and negative segregants of the BC4 generation used in this analysis show faint, but discrete, hybridized bands using the full 'backbone probe', which are not commented by the applicant. Such comments are awaited, and additional Southern blots should be performed, using parental, non-transformed lines as additional negative controls. Insertion of backbone sequences in a different locus could produce such bands in so called BC4 + and – segregants (remind they are named in that way based on the PCR testing of plants using primers corresponding to the transgenes only). The Southern blot analysis could also be complemented with PCR TaqMan analysis with primers deduced from the backbone sequence, especially the antibiotic resistance *aadA* gene of safety concern, using appropriate controls.

2. Potential novel ORFs at the junction regions

The applicant defines a potentially functional ORF as " a region corresponding to at least fifty aa in length …" (see page 6 of CBI-appendix 4) and uses this criterium in the bioinformatic analysis of the junctions between the inserted T-DNA and the target locus. Peptides with biological functions may be much shorter than 50 aa, hence the bioinformatics analysis should be repeated by analysing all possible ORFs of much shorter size (e.g. down to 3 codons). It is a suggestion for EFSA to give

guidelines as to how an ORF is defined and what kind of analysis should be done to prove non functionality of the ORF. Also as small RNA's are getting more in the picture as gene regulators it might be a good idea to set some guidelines. Experts can be asked for their view on this.

3. Regions of homology between the 3'flanking region and maize genomic DNA

The arguments set by the applicant in CBI-Appendix 4 – 'it is not likely that the maize genomic sequence flanking the 3' region of the event 3272 T-DNA insert could function as a promoter as the maize genomic sequence is missing core components necessary for promoter function '- are not very convincing. However, the 'weight of evidence' approach of the GM plants raises no safety concern (no unintended phenotypic effects, see section D7.4 on Agronomic traits).

4. Additional remarks and questions

P 16: the PEPC9 intron is located after the stopcodon of the AMY797E sequence. What is its function? Why is it there?

P18: Blast analysis of the 5' and 3' T-DNA flanking sequences: There is no information on how these sequences were obtained (inverse-PCR, genome walking, …?).

Can a conclusion be drawn about the chromosomal location of the T-DNA insert (on which chromosome)?

Although some short sequence similarities are found with the 3' sequences (eg chromosomal maize sequence AF391808 and BAC clone c573L14) one can ask the question if these are significant. Why are not similarities found with the 5' sequence since the T-DNA is supposed to insert at one locus. Why are only short sequence similarities found with the 3' sequences and not sequence identity with the complete 1000 bp flanking sequence? Could it be possible that these short sequences with high sequence identity are due to the presence of genome duplications in the maize genome? Should the conclusion be drawn that the sequence surrounding the T-DNA insert is not yet available in Genbank? Conclusion: it would be informative to add more data about the T-DNA flanking regions

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments submitted on the EFSAnet

Validation of the ELISA tests

The dossier presents expression data of both the AMY797E and PMI proteins based on ELISA (CBI Appendix 5). No western blot on the corresponding tissue extracts is shown for the validation of the test, especially for the AMY797E polyclonal antibodies which could show some cross reactivity with endogenous amylases. Such possible cross reactivities are underlined by the applicant (page 27 of the Technical dossier) but not taken into account by the immunoassays. CBI Appendix 10 shows western blots, but on the immunoaffinity purified AMY797E test sample only, regrettably not on plant extracts. Moreover, the maize line used for preparing the test AMY797E-0104 is described in vague terms – 'from transgenic maize grain derived form event 3272' (page 8 of Appendix 10) – and the applicant should be more accurate on this and assure that the test substance corresponds well to the plant materials used in the ELISA tests.

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

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 submitted on the EFSAnet

In the literature, it is claimed that crude corn oil is/will be extracted from distiller's grain derived from dry mill ethanol factories [1]. Although this oil is said to be intended for biodiesel production [1], it can not be excluded that it comes into the food chain. Therefore, compositional analysis of such oil derived from genetically modified grains and comparison with that from conventional corn oil may be warranted**.** In the dossier it is stated that the end products of Event 3272 maize grain after the dry-grind ethanol process are not different from those obtained from conventional corn but no data are given.

Q: Are there data on the composition of distiller's grain derived corn oil from Maize 3272 compared to that from distiller's grain from conventional corn?

Low concentrations of AMY797E alpha-amylase were found in the non-transgenic near-isogenic controls (Technical dossier page 31**).**

Q: Were these controls obtained from field trials in the proximity of the genetic modified plants where there could have been gene transfer or is it really "contamination"? If so, why than no contamination with commercially-sourced grain?

Cryptoxanthin levels are expressed as retinol equivalents (which is one way to do this) (Technical Dossier, Appendix A, Vol 1 pp 26) but the literature reference value in microgram/100 g. No

convertion factor for mass cryptoxanthin in retinol equivalents is given however . Such a factor is required to see whether the found values are within the literature range as stated. Note that there is a discussion amongst nutritionists what conversion factors of weight to RE would be required for retinoids [2]. Further note that there are more recent papers on cryptoxanthin levels in corn [e.g. 3,4] and that there is a tendency to express carotenoids in weight/ g [5].
Q: What was the convertion factor used to convert weight content of cryptoxanthin into retinol equivalents?

D.7.2 Production of material for comparative assessment

Comments submitted on the EFSAnet

AMY was extracted from Event 3272 transgenic maize which is a favourable aspect with regard to the selection of the material for analysis. However, phosphomannose isomerase (PMI) was produced by recombinant E. coli, because it may be practically impossible to obtain a sufficient amount of plant derived protein. Although the PMI proteins from recombinant E. coli and from Event 3272-derived maize were determined to be substantially equivalent (Technical dossier, p.27), it has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and Schubert, 2004).

D.7.3 Selection of material and compounds for analysis

Comments submitted on the EFSAnet

None

D.7.4 Agronomic traits

Comments submitted on the EFSAnet

None

D.7.5 Product specification

Comments submitted on the EFSAnet

None

D.7.6 Effect of processing

Comments submitted on the EFSAnet

None

D.7.7 Anticipated intake/extent of use

Comments submitted on the EFSAnet

None

D.7.8 Toxicology

Comments submitted on the EFSAnet

None

D. 7.8.1 Safety assessment of newly expressed proteins

Comments submitted on the EFSAnet

Technical dossier P 28: concerning the stability of the PMI protein I would prefer to change the following text:
"… the results of this study showed that the PMI protein is essentially inactivated after incubation at 65°C…" into "… PMI protein activity is strongly reduced (eg 2% residual activity) after incubation at 65°C…"

D.7.8.2 Testing of new constituents other than proteins

Comments submitted on the EFSAnet

None

D.7.8.3 Information on natural food and feed constituents

Comments submitted on the EFSAnet

None

D.7.8.4 Testing of the whole GM food/feed

Comments submitted on the EFSAnet

None

D.7.9 Allergenicity

Comments submitted on the EFSAnet

Assessment of allergenicity of the newly expressed proteins
As mentioned by the applicant, AMY797E and PMI are not likely to be allergenic. The homology searches with known allergens use a 2005 database release in the AMY797 study, and a 2006 release for the PMI study. Authors are requested to use the latest releases for all studies, hence to uptade the AMY797 study.

Assessment of the allergenicity of the whole GM plant or crop
In section 7.9.2, the allergenicity of the genetically modified maize itself has not been evaluated. The rationale of this section is not to take the new traits into consideration, but to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source.
Although not frequent, food allergy to maize exists and major allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002), and new allergens might be described in the near future (Weichel et al. 2006). The introduction in the plant of AMY797E and of phosphomannose isomerase and the effects thereof might interfere with the expression levels of other maize proteins, including allergens. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified 3272 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize can be carried out.

FAO/WHO (2001) proposes pepsin degradation as a method for the evaluation of allergenicity of genetically modified foods. However, it is not necessarily safe to conclude that a protein that degrades rapidly in simulated gastric fluid is not an allergen. Furthermore, the similarity of amino acids with know allergens was studied as described by FAO/WHO (2001), where a cross-reactivity between the expressed protein and a known allergen hasto be considered when there is:
1) more than 35 % identity in the amino acid sequence of the expressed protein, using a window of 80 amino acids and a suitable gap penalty, or
2) identity of 6 contiguous amino acids. In this dossier, a sequence homology of 8 contiguous amino acids was used, while a six amino acid match is more appropriate and would avoid any false negatives. However, there is no proof that a six or eight amino acid match is predictive in the bioinformatics section. A number of people now recommend not performing the 6-8 amino acid match. There was one region of sequence homology of eight contiguous identical amino acids between PMI and a known allergen, alpha-parvalbumin from *Rana species* CH2001. For the abovementioned reason, it may a false negative.
Simulated gastric fluid (SGF) was used to test the digestion of AMY and PMI proteins The fact that major allergens with high percent allergenicity were not necessarily more resistant to SGF or SIF digestion than allergens with low percent allergenicity renders the use of SGF and SIF digestibility difficult as a tool to distinguish potential food allergens from nonallergenic proteins (Fu et al., 2002). Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. An additional issue would be the physiological relevance of the test. In reality, protein does not enter the acid environment of the stomach as a

pure test solution, but rather as part of a complex food matrix. Within a bolus of food passing through the stomach, it is unlikely that all protein is exposed to the extremes of acid pH, and some protein is likely to survive intact into the lower intestine. One of the properties of food allergens is their thermal stability (Breiteneder and Mills, 2005). PMI is active at ambient temperatures and at 95°C for 30 minutes (Appendix 13). AMY was specially selected for its thermal stability. However, details of the thermal stability are lacking in the dossier in contradiction with PMI.

D.7.10 Nutritional assessment of GM food/feed

Comments submitted on the EFSAnet

No information dealing with the in vitro organic matter digestibility of Event 3272 maize was found. This is a rapid technique that can provide interesting information: based on the chemical composition and the vitro organic matter digestibility, the metabolic and net energy can be estimated, yielding extra information for pigs and ruminants. In the poultry feeding study, feed efficiency was not different, which may be an indication of a similar digestibility of GM and control maize. It would be interesting to know the effect of AMY in maize grain on starch degradability in the stomachs of ruminants. If the starch is more rapidly degraded in the presence of AMY, it may provoke rumen disorders in diets containing a high amount of maize which may be detrimental.

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments submitted on the EFSAnet

None

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments submitted on the EFSAnet

None

D.11.2 Interplay between environmental risk assessment and monitoring

Comments submitted on the EFSAnet

None

D.11.3 Case-specific GM plant monitoring

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

Although general surveillance of potential, long term, indirect effects poses methodological difficulties, the applicant is too vague on the nature and qualities of the participants of this surveillance, despite the fact that an annual report of the general surveillance activities is announced, complying to the EFSA guidelines. Relying on "an adequate number of people, with relevant experience" (page 4 of Appendix 25) is not precise enough. The applicant should elaborate on this.

D.11.5 Reporting the results of monitoring

Comments submitted on the EFSAnet

None