Bioveiligheidsraad
Conseil de Biosécurité

21-05-2014

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0329

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2007/46 and EFSA/GMO/RX/T25 from Bayer CropScience AG under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2007/46 was submitted by Bayer CropScience on 24 April 2007 within the framework of Regulation (EC) No. 1829/2003[1] for the marketing of genetically modified maize T25 for food and feed uses, import and processing and cultivation. Maize T25 carries a *pat* gene conferring tolerance to glufosinate-ammonium herbicides.

The application was officially acknowledged by EFSA on 10 June 2008. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 8 September 2008.

The opinion of the EFSA GMO Panel was adopted on 11 September 2013 (EFSA Journal 2013; 11(10):3356[2]), and published together with the responses from the Panel to comments submitted by the experts during the three-month consultation period.

On 9 October 2013 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

On 29 June 2007, EFSA received application EFSA-GMO-RX/T25 under Regulation (EC) No 1829/2003 for renewal of the authorisation of feed produced from maize T25. It was officially acknowledged by EFSA on 9 October 2008. The formal three-month consultation period of the Member States started on the same date. The Biosafety Advisory Council did not take part in this consultation considering that the comments raised in the frame of application EFSA/GMO/NL/2007/46 were also valid for the renewal application.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/3356.htm

On 14 January 2013 the applicant requested to modify the scope of EFSA/GMO/NL/2007/46 to no longer include cultivation of maize T25 in the European Union. Therefore, the present advice of the Biosafety Council only covers the evaluation of the potential effects on human and animal health or the environment of maize T25 in the context of import and processing and its use as food and feed.

Scientific evaluation

1. Environmental risk assessment

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

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The Biosafety Advisory Council is of the opinion that the composition of the GM maize T25 is compositionally equivalent to its non-GM counterpart and conventional maize varieties.

The Biosafety Advisory Council also considers that, although not required by the OECD document on compositional considerations for new varieties of maize (OECD, 2002), it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies.

3.2. Assessment of toxicity

The toxicological assessment of the PAT protein has been done by the BAC in the context of several previous applications and no concerns were identified

With regard to toxicity of the whole GM food/feed, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed PAT protein has been assessed in the context of this application but also in the context of several previous applications[i]. No concerns in relation to allergenicity were identified.

With regard to the allergenicity of the whole GM plant, maize is not considered to be a common allergenic food. Moreover additional information was provided by the applicant in the form of a skin prick test with protein extracts from maize T25. The test showed similar reactions to the GM maize as for non-GM maize.

[3] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment was not performed.

Based on the available information, the Biosafety Advisory Council considers that there is no evidence that the overall allergenicity of maize T25 is changed as a result of the genetic modification.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and shows the nutritional equivalence of the GM maize with its non-GM counterpart and conventional maize varieties.

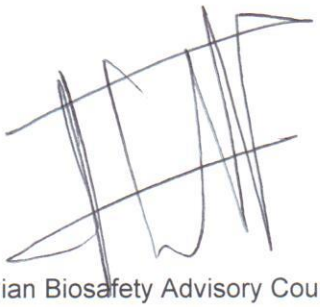
4. Monitoring

Since the allergenicity of the whole GM maize has not been fully assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the questions of the EFSA GMO Panel and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM maize T25 is unlikely to pose any risk to human and animal health or to the European environment.

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



23-5-2014

Prof. M. De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2007/46 and *comments submitted on the EFSAnet (ref. BAC_2008_805)*

Wetenschappelijk Instituut Volksgezondheid | Institut Scientifique de Santé Publique
Dienst Bioveiligheid en Biotechnologie | Service Biosécurité et Biotechnologie
Rue Juliette Wytsmanstraat 14 | B-1050 Brussels | Belgium
T + 32 2 642 52 11 | F + 32 2 642 52 92 | bac@wiv-isp.be | www.bio-council.be

Bioveiligheidsraad
Conseil de Biosécurité

08/09/2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2008_805
Email. : bac@sbb.ihe.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2007/46
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 June 2008
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Pascal Cadot (Consultant), Eddy Decuypere (KUL), Patrick du Jardin (FUSAGx), Leo Fiems (ILVO), Godelieve Gheysen (UGent), Jean-Pierre Hernalsteens (VUB), André Huyghebaert (UGent), Peter Smet (Consultant)
Domains of expertise of experts involved: Genetics, genome analysis, molecular characterisation, genetic engineering, transgene expression, human nutrition, animal nutrition, biochemistry of food/feed, analysis food/feed, substantial equivalence, toxicology, general biochemistry, immunology, alimentary allergology, agronomy, herbicide tolerance, risk analysis, monitoring, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/NL/2007/46** concerns an application of the company **Bayer CropScience** for the marketing of the genetically modified **maize T25** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 10 June 2008.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☒ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in

the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

From p.64 onwards the heading is changed into “1829/2003 LLCotton25 – Part I – Technical Dossier”. We hope that parts of a previous dossier, dealing with cotton, were deleted and that the text was the original text for T25 maize.
T25 maize has been introduced for commercial use for several years in some parts of the world. Up to now, there are no indications of adverse effects.
With regard to PAT proteins, no identity with known allergens was shown, and no concerns were raised with regard to toxicity.

Comment 2

The pat-gene encodes for the enzyme phosphinothricin acetyl-transferase (PAT) which acetylates glufosinate ammonium to its inactive form and thereby detoxifies the herbicide; therefore, when the pat gene is expressed it confers to the tolerance of the mentioned herbicide. The enzyme is so highly substrate specific that it acts on its target glufosinate but not on glutamate which is the closest structural analogue of L-glufosinate.

Comment 3

No comment

Comment 4

none

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

The data given correspond to the very well known properties of maize as a crop plant.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

Complete information is given on the DNA sequence of the vector DNA.

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

Description of the trait: see A, no questions.

Comment 2

No comment

Comment 3

none

Comment 4

The inserted DNA encoding the PAT enzyme has been used in numerous applications in plant genetics and biotechnology and is well understood. Its only influence on the phenotype of the plants is to confer herbicide resistance.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Editorial change (on several places including page 42 of the technical dossier : *The first 5bp of the bla gene (containing the translation* – instead of "transcription" – *initiation codon ATG)*.

Comment 3

Besides the numerous language errors ('maze instead' of maize, 'lost of' instead of 'loss of',....) that we do not want to go in detail to (as long as the text is understandable), some words need to be adjusted to be correct:
In technical dossier:
- p32 and p42 should be 'translation initiation codon' instead of 'transcription initiation codon'.
- p32: Concerning the piece of pUC/Ac DNA that is integrated, we deduce from the sequence (Berghman and De Beuckeleer, 2000a,b) and the graph that the inserted part should be from bp 3779 instead of 3814 and bp 3555 should be adapted to 3587.
- Furthermore, although the sequence analysis and the provided Southern blots are convincing to show the characteristics of the inserted DNA, the PCR results shown in the reports are not at all clear. We do not believe that this makes a difference for the safety evaluation of the GM plant, but we do not see what the authors claim to see on the pictures.
More specifically in the following reports: De Beuckeleer 2003a and, Aerts and De Beuckeleer 2003. The figure provided in the former report is totally obscure: one can only see (but very clearly) 2 PCR-fragments of 513bp (although an arrow points to invisible 202 bp fragments) in lanes that according to the legend correspond to event X and wild type maize. Why is this fragment not seen in event T25 (is it disrupted by the transgene insert?)? According to the other report, it should be there however.
The latter report has several unclarities. 6.1.1. claims a 513 bp fragment by the same primers as above to generate a fragment in all maize lines (lanes 2-4), one can only see a band in lane 3. The 202 bp fragment mentioned in the text is not visible on the picture. 6.1.2.: problem with the 513 fragment as above for 6.1.1.

Additional comment from the SBB

In Berghman and De Beuckeleer, 2000a:
- b.) Description of the genetic elements of pUC/Ac: as pUC/Ac has a size of 3982 bp, 6411 (last row of table) is incorrect.

Comment 4

The DNA insert present in line T25 was studied in detail. The data that are presented support the conclusions on the structure of the insert.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Section (a) on the expression of PAT : EFSA guidance on application for existing products (EFSA Journal (2006) 435, 1-4) asks applicants to update the information on the expression levels of the specific proteins. However, in the updated information shown by Dewulf and Pestel, 2007, three tissue sources only are used – leaves, stems, roots – and no more information on expression in grains and pollen is presented. Although no detectable PAT activity was shown by Klonus et al 1999 in pollen, activity was measured in seeds. The applicant should be requested to present PAT protein content data for leaves, roots, stems + seeds and pollen. Not only activity measurements (like in Klonus 1999), but also PAT protein contents should be provided.
Furthermore, when comparing the leaf measurements of Deschamp (1996) with those of Dewulf and Pestel 2007, it comes out that the levels measured by the latter are significantly lower than those provided by the former : ca 25-50 microgram /gram (see page 16 of Dewulf and Pestel 2007) *vs*. 0.9 – 1.3 microgram / gram (see page 42 of technical dossier). I understand that developmental stages are similar (V5-V6 and mature leaf in Dewulf and Pestel, “Leaf 6 mature” in Deschamp) and the applicant is requested to comment on this apparent discrepancy.

Overall, the expert is of the opinion that the updated information on PAT protein expression levels in plant tissues is not satisfactory and additional data and explanation should be asked to the applicant.

Additional comment coming from comment under D.7.8

PAT protein measured in T25 (De Wulf and De Pestel, 2007).
Please provide data based on dry weight.

Comment 3

none

Comment 4

I agree with the conclusions of the study: only the PAT protein will be expressed and specifically confer resistance to the herbicide phosphinothricin.
In bacteria, the intact beta-lactamase gene can confer resistance to several penicillin-type antibiotics. However the fragment of the gene that is present in the T25 transgenic maize is non-functional and is moreover also not expressed. Even the expression of the complete enzyme would not influence the phenotype of the plants, as its substrate is not present in plants.

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 questions

Comment 2

In line with the EFSA guidance document (section III D 7.2), where herbicide tolerant GM plants are to be characterized at the agronomic level, field trials should use both glufosinate-treated and non treated plants (i.e .no glufosinate treatment but treatment with conventional herbicides), in comparison with non GM near isogenic lines (treated with conventional herbicides). With the exception of the annexed reports of Oberdoerfer 2002 (a, b and c) and 2004 (a and b), all the tables 11 to 16 of the technical dossier and all quoted reports either show glufosinate-untreated GM plants only or do not specify anything. In consequence, data should be presented corresponding to plants treated with glufosinate ammonium according to the intended usage of the herbicide in the agronomic practice before any firm conclusion may be drawn on the agronomic behaviour of the GM lines.
Among the developmental traits investigated, time to tassel development and time to anthesis were both reported (page 46 and 80 of the Technical dossier + Oberdoerfer, 2004b), showing some deviation from the non GM control in some trial locations. However, interpretation is complicated by the fact that in Oberdoerfer, the tested varieties containing T25 differ from the varieties mentioned in Table 11.
Oberdorfer 2004 a mentions (p12/194): The test substance is the gene event T25. The transgenic, glufosinate-tolerant maize event
T25 of the cultivars LL Moldova and LL Kingston contains the pat gene that expresses the phosphinothricin acetyltransferase (PAT) protein. The name of the herbicide formulation is Liberty®. For comparison non-transgenic, maize of the cultivars Cecilia and Torino were used.
Table 11 mentions completely different varieties !

The notifier should clarify this.

Comment 3

none

Comment 4

On the basis of the molecular analysis, the only difference between the transgenic plants and isogenic non-transgenic plants that can be expected is herbicide resistance. This is supported by the results of the field tests.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

The data that are shown support the stability of the inserted sequence during mitosis and meiosis.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

The only realistic possibility to transfer genetic material to other organisms is crossing with other maize plants. This is not affected by the genetic modification.

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

No comments

Comment 2

Composition analysis of grain (Oberdoerfer, 2006b, Rattemeyer-Matschurat, 2006b).

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

Vitamins		**Amino acids**		**Fatty acids**		**Secondary metabolites**		**Antinutrients**	
A (β-carotene)	X	alanine	X	8:0 caprylic	X	ferulic acid		phytic acid	X
B1 (thiamine)	X	arginine	X	10:0 capric	X	furfural		raffinose	
B2 (riboflavin)	X	asparagine	X	12:0 lauric	X	inositol		trypsin inhibitor	X
B3 (niacin)	X	aspartic acid	X	14:0 myristic	X	*p*-coumaric acid		gossypol	
B4 (choline)	X	cysteine	X	14:1 myristoleic	X			malvalic acid	
B5 (pantothenic a)	X	glutamic acid	X	15:0 pentadecanoic	X			sterculic acid	
B6 (pyridoxine)	X	glycine	X	15:1 pentadecenoic	X			dihydrosterculic acid	
B9 (folic acid)	X	histidine	X	16:0 palmitic	X				
C (ascorbic acid)		isoleucine	X	16:1 palmitoleic	X				
E (α-tocopherol)	X	leucine	X	17:0 margaric	X				
Cryptoxanthin	X	lysine	X	17:1 heptadecenoic	X				
		methionine	X	18:0 stearic	X				
		phenylalanine	X	18:1 oleic	X				
		proline	X	18:2 linoleic	X				
		serine	X	18:3 linolenic	X				
		threonine	X	20:0 arachidic	X				
		tryptophan	X	20:1 gadoleic	X				
				20:2 eicosadienoic	X				
				20:3 eicosatrienoic	X				
				20:4 arachidonic	X				
				20:5 eicosapentaenoic	X				
		tyrosine	X	22:0 behenic	X				
		valine	X	22:1 erucic	X				
				22:5 docosapentaenoic	X				
				22:6 docosahexaenoic	X				
				24:0 lignoceric	X				

Proximates, minerals, vitamins, anti-nutrient, amino acids and fatty acids.

There is not a majority of sites at which significant differences between mean values was found; therefore no evidence is given the datasets are not equivalent.

Comment 3

T25 maize was compared with the non transgenic isogenic line. As it is usually the case results were compared with data available in literature.
No further comment.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Trials were designed in randomized complete block with 4 replications, and in all cases (15 different field trials from Europe) samples from non-GM comparators treated with conventional herbicide (A) were compared with samples from T25 plants treated with conventional herbicide (treatment B) and with T25 plants treated with glufosinate ammonium (treatment C). This set-up of the field trials is ideal not only for comparing composition of leafs, stems, seeds of maize, but also for evaluating the effect on soil organisms e.o. This may be of importance in view of a previous observation in the UK Farm Scale Evaluation study where glufosinate ammonium tolerant T25 maize farming system resulted in a greater capture of collembolan detrivores compared to conventional maize herbicide system. It is mentioned that the later application of herbicide and relative abundance of weeds in glufosinate tolerant T25 maize system may be a beneficial indirect effect, and this may well be; however since 2 factors are changed at the same time (the GM-maize and a different herbicide treatment system), a direct effect of the genetic modification on soil life may not be totally excluded. The set-up of these different European field trials using treatment A, B and C can give an exclusive answer to this.

Comment 2

See comment under D4 regarding the necessity of both treated and non treated plant materials in these studies, in parallel to the non GM comparators. This holds true for the agronomic traits, not for the compositional analysis where treated and non treated GM plants were correctly used.

Comment 3

Grain samples of T25 were obtained from 15 different field trials in the maize producing areas within Europe. Trials were performed according to generally accepted procedures.
Each comparison includes a non GMO comparator with a conventional herbicide treatment, a T25 treated with conventional herbicide and a T25 treated with Liberty herbicide.
As a baseline literature data were consulted for the nutritional evaluation.
No further comment.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Studies designed to evaluate the PAT protein for allergenic and toxic characteristics were conducted using highly purified PAT protein produced by Escherichia coli expressing the PAT gene (P. 91 of Technical dossier). With regard to microbial protein, Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins.

Comment 2

- why standard values for calcium (mg/kg) in table 21 (data from appendix A in Oberdoerfer 2002b) are so high ? why minimum value of 100 is twice as high as in the non-transgenic and T25 maize from this study ?
- for the results given in table 27, it is concluded that equivalence between the datasets of the non-transgenic control group and the two transgenic groups can not be excluded. This is a strange way of formulating since the equivalence is rather obvious from the data given.
- Table 35: for NDF (neutral detergent fibre) a NO is given for equivalence evaluation between A/B and A/C, but when taking into account the very big standard deviations (3.16 ± for Non GM maize, versus 3.86 +- 3.19 or 3.50 ± 1.45 for the transgenic maize, sprayed and non-sprayed), I wonder if this is correct ?
- Similar remarks can be made for table 36: no equivalence is mentioned for A/B comparison for fructose (5.51 ± 4.45 versus 5.75 ± 5.84) while on the other hand A/C comparison receives a yes and this with similar differences and even smaller SD (5.51 ± 4.45 versus 5.70 ± 4.97). What then when data of sucrose are compared with maltose for example ? A yes for equivalence between A/B or A/C for sucrose with data 4.58 ± 2.25 versus 3.45 ± 2.17 or versus 2.94 ± 1.45. On the other hand, a NO for equivalence between A/B or A/C for maltose with data 4.04 ± 3.05 versus 3.41 ± 2.74 or versus 3.54 ± 2.70 ?? On the basis of the data given and there SD, I don't understand those equivalence evaluations.
- Table 41. Similar remarks about the NO for equivalency evaluation for A/B and A/C for Calcium, and Iron in view of the small differences of the means and the huge SD: how can there be for example no equivalency between A and C for Iron with means of 19.58 ± 4.76 and 19.98 ± 4.89 ??

Comment 3

No comment

Comment 4

Constituents selected for compositional and nutritional analysis include proximates, amino acids, fatty acids, minerals, vitamins and phytic acid as an anti-nutrient. I agree with the comment that the trypsin inhibitor is not relevant for maize.

Constituents selected for compositional and nutritional analysis include proximates, amino acids, fatty acids, minerals, vitamins and phytic acid as an anti-nutrient. I agree with the comment that the trypsin inhibitor is not relevant for maize.

Proximate analysis includes, in addition to moisture, fat, protein, total dietary fibre, ash, total carbohydrates by difference and available carbohydrates by difference. No significant differences were found with the exception of total dietary fibre. In this case results are somewhat higher than the (rather limited) literature data.
In the expert's opinion these differences are not relevant as the fibre approach has to be adapted to more recent views.
I will not repeat my "fibre" comments as the answer to this remark, by EFSA, is always the OECD document.

Mineral and vitamins cover a broad range of important nutrients: minerals, trace elements and vitamins. With respect to vitamins important constituents are covered including water soluble B vitamins and a range of tocopherols. This is an in depth analysis of minor constituents. Vitamin B6 is added in more recent analysis.
Values obtained are generally within the range of data in literature.

With respect to amino acid analysis, all relevant constituents are covered an no significant differences are found between non transgenic and transgenic samples.
In fatty acid analyses relevant acids are covered including the important polyunsaturated fatty acids. At a population level the contribution of omega-6 fatty acids is important. They are adequately analyzed.

Data are also available about the by site variation of nutrients. Statistical analyses result in most situations in non significant differences.

I agree with the general conclusion about substantial equivalence of grain corn.

Two final remarks concerning this part.
In some maize dossiers other secondary metabolites are studied. I found no data related to these constituents in this dossier.
In a previous dossier I made a comment on resistance to mycotoxin production. Several mycotoxins in maize are contributing to the exposure of humans and animals to mycotoxins. There is no information in the dossier related to this aspect.

For sweet corn, the analyses are somewhat adapted to the particular composition. Fibre analysis is somewhat more elaborated: crude fibre, ADF and NDF are mentioned.
Sugar analysis covers glucose, fructose, sucrose and maltose. Other sugars are below detection limit including raffinose and stachyose. Sugars are adequately studied.
Overall result for sweet corn are in line with those obtained for grain corn.
I agree with the conclusion of substantial equivalence for sweet corn.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

See comment under D4 regarding the necessity of both treated and non treated plant materials in these studies, in parallel to the non GM comparators. See also D 11.5 regarding the surveillance of the agronomic effects after commercial release.

Comment 3

No particular comment.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Why is the PAT enzyme so much higher in sweet maize (whole, fresh maize, table 52) compared to (wet) grain from field maize ?

Comment 2

T25 maize will be processed in the same way as traditional maize.
T25 was processed by a dry milling and a wet milling process. Fractions obtained were analyzed for T25 specific DNA sequences and PAT protein.

No DNA was found in the refined oil samples. The applicant demonstrated that DNA is degraded during processing of the crude oil. This is quite acceptable taking into account the normal processing conditions.

The PAT protein was detected in dry milling fractions. The crude oil and oils samples taken during refining did not contain the PAT protein. The presence of the PAT protein was demonstrated in other dry milling fractions: bran, germ, grits, meal, flour and defatted germ meal.

A similar study was performed for the wet milling process. Taking into account the better separations obtained by this process the PAT protein was only detected in pressing germ cake, the toasted germ meal and the extraction germ meal. No PAT protein was detected in other fractions like hulls, starches, gluten, pressing crude oil, extraction crude oil and refined oil .

For sweet corn the distribution (or degradation) of PAT protein was studied in fresh maize, frozen, canned, canned creamed and cannery by products. It was demonstrated that the PAT protein was present after processing, even after canning.

Both studies are in my opinion exemplary for a study on the effect of processing.
I have no further comment but I found this part a very interesting one.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Maize T25 is intended to replace part of the maize in the supply chain. There are no anticipated changes in the intake or extend of use of maize as the result of the presence of T25.
No further comment.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

The amount of PAT protein present in T25 maize is small: ±30 ng/g (0.00002% to 0.00025% of total crude protein). Acute exposure in mice at 10 mg/kg body weight by the intravenous route did not result in a detrimental effect, so that the safety risk may be very low.
Furthermore, none of the milk samples analysed in an experiment of Phipps et al. (2005) was positive for T25 maize tDNA (above a detection limit of 2.5 ng of total genomic DNA/ml of milk).

Comment 2

Safety of the newly expressed PAT-protein based on:
- no sequence homology with known allergens or toxins
- not heat stable
- not stable in digestive environments
- no toxic signs after acute intravenous injection of very high dose in mice
- no subchronic oral toxicity

Testing the whole GM feed, it was stated that the feeding study was performed on male broilers over an entire life span and under conditions of rapid growth.
We suggest to delete "over an entire life span", as the biological life span of poultry is several years, but the economic life span of a broiler is ± 6 weeks; and in the study of Leeson (1996) male broilers were used.

Comment 3

Mean concentrations of:

a) PAT protein measured in T25 (De Wulf and De Pestel, 2007).

Growth stage/ Tissue	**ng/mg Tissue Fresh Weight**		**Standard deviation**
	Mean	**Range**	
Leaves (V5-V6)	24.6		5.92
Leaves (Seed Maturity)	41.99		8.12
Stem (V5-6)	1.50		0.31
Stem (Seed Maturity)	2.85		0.86
Roots (V5-V6)	2.06		0.32
Roots (Seed Maturity)	1.75		0.40
Kernels (Seed Maturity)			

Please provide data based on dry weight.
Do kernels contain the PAT protein? If so, why isn't this mentioned in the study?
Data are provided in Deschamps, 1996a. These do not seem directly comparable with the data above. When comparing the above values with those of other GM maize, it seems that the PAT content is much higher in this application. Is this correct? What is the cause of this and what benefit does it bring along?

See comment under D.3

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

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

Rapid degradation - within 30 seconds - of the PAT protein (encoded by the pat gene) in simulated gastric fluid (SGF), in the presence of pepsin, at pH 2,0. The degradation was caused by the pepsin present in the SGF.

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

Results showed almost immediate degradation of the PAT protein in simulated intestinal fluids (SIF) (pH 7.5), in the presence of pancreatin. The residual fragments, at about 5 to 14 kD, completely disappeared in less than 30 seconds of incubation and were not detectable with the Western Blot method. The degradation was linked to the presence of pancreatin in the SIF.

c) PAT: Acute Oral Toxicity Study in Mice (Kennel, 2003).

An acute intravenous toxicity study was conducted in mice with PAT protein, encoded by the *pat* gene, produced in *E. coli* and highly purified (>90%). Groups of 5 female mice were administered either PAT protein, aprotinin (negative control) or melittin (positive control) at dose levels of 1 and 10 mg/kg body weight. All animals were observed for clinical signs daily for 15 days while their body weights were measured weekly. At study termination, animals were subjected to a necropsy including macroscopic examination.
The results showed that the animals treated with the PAT protein and aprotinin at 10 mg/kg had no visible signs of systemic toxicity, by contrast to melittin which induced 100% mortality within 5 minutes at the same dose. Based on these results, it is concluded that the PAT protein is very unlikely to be toxic to humans or mammals even under conditions of maximal exposure at a very high dose (10 mg/kg body weight) by the intravenous route.

d) Repeated dose oral toxicity (14-day feeding) study in rats (Pfister *et al.*, 1999).

In this repeated dose oral toxicity study PAT protein was administered by feed admixture in powdered diet to Wistar rats at concentrations of 0 (group 1*), 5000* (group 2), 50000 (group 3) and 0 ppm (group 4) for a period of 14 days.
Animals of group 1 received a standard diet and rats of groups 2, 3 and 4 a low protein diet, which was adjusted to a protein content similar to that of group 1 by using soya bean derived protein at concentrations of 45000 ppm for group 2, 0 ppm for group 3 and 5000 ppm for group 4. The study comprised four groups of each five male and five female rats.

group	Average intake of PAT	
	Males (mg/kg/day)	Females (mg/kg/day)
1	0	0
2	712	703
3	7619	7965
4	0	0

The study with the PAT protein encoded by the *pat* gene showed no adverse effect. No unscheduled mortality and no clinical signs were observed in any group. Food consumption and body weights were not affected by the treatment.

e) Amino acid sequence homology with known toxins (Hérouet-Guicheney, 2006b).

The overall homology search with the PAT protein (*pat* gene product) showed no evidence for any similarity to known toxins. Recent results of the in silico analysis showed no evidence for any similarity between the PAT proteins (*bar* or *pat* coding sequence) and any known toxic protein. The results also showed that the PAT proteins only have high structural similarity with other acetyltransferase proteins, for which no adverse effects have been reported following consumption.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comment.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

The composition of grain maize and sweet maize are studied in depth, see above.
No further comment.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Total dietary DM consisted of 39% maize silage, besides grass silage and concentrate in the experiment of Phipps et al. (2005). In European dairy and beef farms, maize silage may be included at higher levels in the diet of dairy cows and other ruminants. It would be usefull to have information of diets composed of higher shares of maize silage.

Comment 2

a) 42-day feeding study with broiler chickens (Leeson, 1996).

There was no effect on body weight, feed intake, body weight gain or percent mortality over the experimental period (P >.05).

b) 90-day rat feeding study ().

Not performed. But such a test is highly recommended.
No further testing is needed. Adinda: most members of the biosafety council (including the food expert whe invited during the last meeting !!!!) are of the opinion that a 90-day feeding test IS necessary. What do we do with this comment ?

c) Effect on milk production and absence of transgenic DNA in milk (Phipps et al., 2005).

The study showed no significant differences between the 4 dietary treatments for milk yield, milk composition, and yield of milk constituents. In particular, there were no significant treatment effects when comparing T25 with the near-isogenic nonGM variety (T2). In addition, there was no significant difference in dry matter intake when comparing the GM variety T25 and the 2 commercial varieties, Fabius and Antares. However, although the dry matter intake for the near-isogenic nonGM counterpart was similar to the commercial varieties, it was significantly lower *(P* = 0.013) when compared with the GM variety. In tegenspraak met bovenstaande zin !

During the experimental feeding period, there was no significant difference in live-weight gain when comparing the GM variety T25 and the 2 commercial varieties. However, the live-weight gain recorded for the cows receiving the nonGM near-isogenic counterpart was significantly higher *(P* < 0.001) compared with the GM variety.

In the pretreatment and treatment periods, none of the milk samples analyzed was positive above a detection limit of 2.5 ng of total genomic DNA/mL of milk for either tDNA (event T25) or the endogenous corn gene (alcohol dehydrogenase).

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Although microbial surrogate protein has been used, a series of toxicological studies were conducted to evaluate the safety of T25 maize:
- the similarity of amino acids with known allergens was studied as described by FAO/WHO (2001), where a cross-reactivity between the expressed protein and a known allergen has to 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. Here, the total amino acid sequence of the PAT protein was subdivided into 8 amino acid blocks for comparison with known allergens, based on methods of Hilleman et al. (2002).

- almost immediate degradation of the PAT protein occurred in simulated intestinal fluids; however, 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 and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.
- A rapid degradation of the PAT protein occurred in simulated gastric fluid; however, a rapid in vitro digestion is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005).

The combination of all these analytical approaches means that the chance for allergenic reactions may be very low.

Comment 2

No comments

Comment 3

Assessment of the allergenicity of the newly expressed proteins.
According to currently available data, PAT is unlikely to be allergenic.

Assessment of the allergenicity of the whole GM plant or crop.
The applicant did not assess the allergenicity of the whole GM plant. Conversely to what is stated in the application, food allergy to maize has been documented, although it is not recognized as a major allergy concern. Some maize allergens have already been described in the literature (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006).
Due to the introduction of the new trait as described in the application, over-expression of endogenous proteins, among them possibly the maize allergens, may occur. Therefore, it is relevant to analyze whether the expression levels of known maize allergens is increased in genetically modified T25 maize grains or to analyze whether the overall allergenicity of the modified maize grains has increased, compared to a natural counterpart. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out.
The applicant refers to a publication by Batista et al (2005) to claim the non-increased allergenicity of T25 maize grains. In this publication, skin-tests with maize grains extracts were conducted on a general asthma-rhinitis population (n=50), some of them only being reactive to maize. These patients did not show extra-skin-test reactivity to T25, as compared to controls, which indicates that T25 is not more allergenic than control maize.
For the application, however, it is also relevant to analyze IgE-binding to T25 grain extract of maize allergic patients in order to detect individual and possible new specificities. This was not carried out in the quoted paper, conversely to what is claimed by the applicant.

Since the application also deals with cultivation in the EU, respiratory allergy to maize pollen is an issue that must be investigated. Although literature on that subject is scarce, allergy to maize pollen is well known in the allergy outpatient departments of the clinics and of the independent allergologists. It results from cross-reactivity with grass pollen, and is a major allergy problem in children living near maize fields. The most known cross-reacting allergens are Zea m 1 and Zea m 13, that cross-react

with the group 1 and 13 allergens of grasses (Petersen et al. 2006). Therefore, the expression level of those major allergens should be determined in the pollen of genetically modified maize T25, compared to a non-modified counterpart.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

It is remarkable that the asparagine content in Table 24 for T25 maize (B, Transgenic: 1.456 g/kg dry matter) is approximately 3 times higher than in (A, non-Transgenic maize: 0.447 g/kg). Looking at the sum of amino acids, 4.020 g was obtained compared to 2.90 in Table 24. Nevertheless, the values correspond with those reported by Oberdoerfer (2002b; Non-confidential appendices, Table 4.8.4). Is 1.456 the real content ?Or is it a mistake and should it be replaced by 0.456g/kg?

Table 38 shows a starch content of 2.8±1.7 for C (T25 maize), compiled for the 5 sites: this seems unacceptable, looking at individual analyses for the different sites.

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

It is stated that food or feed derived from T25 does not present ethical or religious concerns and does not require special labelling beyond that required under regulation 1830/2003.
I propose to omit the term “ethical” here for the following reason: it is not because the pat-gene in T25 maize is not from human or animal origin, or because no differences in composition except the PAT-protein, or no differences in food or feed value are found, absence of toxicity or allergenicity … that there may be no ethical concerns. Ethical concerns may arise from a certain view on nature and human impact on it, based on subjective reasons originating from such a view, and not only based on objective arguments or safety.
Even if these arguments giving rise to ethical concerns are completely subjective, the ethical concerns are nevertheless real and have to be taken into consideration in a democracy if they arise in a substantial part of the population.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

none

Comment 3

I agree with the dossier. The transgenic plant has no target organisms.

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

As pointed under D.4., it is unclear if the study of the phenotypic and agronomic traits is conducted with appropriate plant materials (treated GM plants).

Comment 3

none

Comment 4

Maize will not survive in nature without human intervention. This will not be changed by the genetic modification. The herbicide resistance gene will only influence the survival of the plants if these are treated with phosphinothricin.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

As pointed under D.4., it is unclear if the study of the phenotypic and agronomic traits is conducted with appropriate plant materials (treated GM plants).

As pointed under D.4., study of the phenotypic and agronomic traits lacked appropriate plant materials (treated GM plants).

Comment 3

none

Comment 4

Plants with the T25 insert would only have a selective advantage if phosphinothricin or a related herbicide is applied. This has no significant implications, as maize will not survive in nature without human intervention.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

Only crossing with other maize plants, possibly resulting in transfer of the herbicide resistance trait is relevant.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

The transgenic plant has no target organisms.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

none

Comment 4

The transgenic plant has no target organisms.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

The chance that T25 maize may exert detrimental effects on animal health is very limited.

Comment 2

No questions

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

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

Comments/Questions of the expert(s)

Comment 1

Table 54 as mentioned on p. 102 should be table 58.

As mentioned already under D7.2, the experimental set-up of these 15 fields trials would have been ideal to test the environmental benefits in an unequivocal way.
Now the mentioned indirect benefits of T25 GM-corn as observed in the 3 years program of the Farm Scale Evaluations in the UK are ascribed to the herbicide management of the weed control.

Indeed, unlike with a non-GM corn, in a field of GM corn, weeds are present in the field for most of the season except at the point when the corn is sensitive to competition from weeds.
This provides the basis for a higher abundance of collembola, an a tendency for more soil surface active invertebrates in field margins.
However, although very plausible as indirect environmental benefits from the new GM-crop farming practice, scientifically 2 variables were taken together in this comparison so that in theory no distinction can be made between the direct effects of the genetic modification “per se” and the indirect effects linked with the alternative herbicide treatment.
In other words, in the UK study GM + glufosinate is compared with non-GM + atrasine and Bromoxynil.
The fields trials as described here are comparing non-GM corn + conventional herbicide with GM corn + conventional herbicide as well as GM corn + conventional herbicide with GM corn + glufosinate and therefore are perfectly suited for a comparison of the direct and indirect effects separately.
However, the environmental impacts such as weed density and weed spectrum, soil surface invertebrates etc. are not studied or followed here in these 15 trials as far as I have the information about this in the technical dossier. Why is this ? Is this not a missed opportunity ?

Comment 2

No comment

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comment

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comment

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comment

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No comment

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Shortcomings in the evaluation of the agronomic properties of the GM plants have been underlined (lack of both treated and non treated GM plants in the field evaluations). The surveillance plan including the farm questionnaire (Farm Questionnaire, 2007) addresses non anticipated agronomic effects. Careful reporting on this is essential if additional data can not be provided before the marketing consent.

References

Bannon,G., Fu, T.J., Kimber, I., Hinton, D.M. (2003). Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124.

Batista et al. (2005) Lack of detectable allergenicity of transgenic maize and soya samples. J Allergy Clin Immunol 2005; 116:403-10

FAO/WHO, (2001). Evaluation of allergenicity of genetically modified foods: Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. FAO, Rome, 27pp. [http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf].

Freese, W., Schubert, D. (2004). Safety testing and regulation of genetically engineered foods. In Harding, S.E. (Ed.) Biotechnology and Genetic Engineering Reviews 21: 299-324.

Herman, R.A., Storer, N.P., Gao, Y. (2006). Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154-1157.

Hileman, R.E., SIlvanovich, A., Goodman, R.E., Rice, E.A., Holleschak, G., Astwood, J.D., Hefleb, S.L. (2002). Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. Int. Arch. Allergy Immunol. 128: 280-291.

Meredith, C. (2005). Allergenic potential of novel foods. Proc. Nutr. Soc. 64: 487–490.

Pasini et al. (2002) IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. *Allergy*, 57:98-106

Pastorello et al. (2003) Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol,* 112;775-83

Petersen et al. (2006) Proteome analysis of maize pollen for allergy-relevant components. Proteomics 2006; 6:6317-25

Phipps, R.H., Jones, A.K., Tingey, A.P., Abeyasekera, S. (2005). Effect of corn silage from an herbicide-tolerant genetically modified variety on milk production and absence of transgenic DNA in milk. J. Dairy Sci. 88: 2870-2878.

Spök, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, W., Valenta, R. (2005). Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int. Arch. Allergy Immunol. 137:167-180.

Weichel et al. (2006) Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. *Allergy,* 61:128-35.