Biosafety Advisory Council

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Secretariat

O./ref.: WIV-ISP/BAC/2006_SC_392
Email: bac@sbb.ihe.be

Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/UK/2004/05** of the company Pioneer Hi-Bred and Mycogen Seeds under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2004/05 was submitted by Pioneer Hi-Bred and Mycogen Seeds in October 2004 for the marketing (import and processing) of the insect-protected glufosinate and glyphosate-tolerant genetically modified hybrid maize 1507xNK603 for food and feed applications under Regulation (EC) No. 1829/2003[1]. It has been officially acknowledged by EFSA on 1st April 2005.

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

In early 2006, the Belgian Biosafety Advisory Council, 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 Biosafety Advisory Council and the Division of Biosafety and Biotechnology. Four experts answered positively to this request They were asked to assess the genetically modified plant considered in the application for (1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. The experts were asked to assess if the information, provided in the application was sufficient to agree that the marketing of the GMO plant for the intended uses, will not raise any problems

[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)

for the environment or human and animal health. In case information was lacking, experts were asked to indicate which extra scientific information is needed.

The comments received from the Belgian experts in April 2006 are stuctured as in the "Guidance document of the scientific panel on genetically modified organisms for risk assessment of genetically modified plants and derived food and feed (EFSA Journal (2004), 99, 1-94. Due to the lack of expertise in the mandated group of experts, some comments are not addressed (see Annex I for an overview of all the comments).

In the meantime, the scientific panel on GMO's (further called the GMO panel) of EFSA adopted an opinion on 28 March 2006, published on 12 May in the EFSA Journal (2006), 355, 1-23[2]. The GMO panel considered all comments submitted by Member States and, when the panel considered it necessary, it requested additional information from the applicant. The GMO Panel concluded that 1507xNK603 is as safe as its conventional counterparts with respect to effect on human and animal health and the environment. The Panel concluded that this maize is unlikely to have any adverse effect on human and animal health and the environment.

On 12 May 2006 EFSA published its opinion on the application: "1507xNK603 fulfils the requirements of Articles 6 and 18 of the Regulation (EC) 1829/2003 for the placing on the market of genetically modified maize.".

The opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react on the GMO panel opinion, specially if, based on their knowledge of the dossier, they estimated that essential points in the dossier have not been taken into account in the opinion of EFSA. None of the experts expressed negative reactions on the GMO panel opinion.

Specificities on the GMO variety

Maize hybrid 1507xNK603 is a single cross hybrid between the two inbred lines line 1507 and line NK603. Both lines are genetically modified.

a) The maize inbred line 1507 was developed to be tolerant to the herbicide glufosinate by the introduction of a gene from the *Streptomyces viridochromogenes* (an aerobic soil micro-organism). This *pat* gene encodes an enzyme phosphinothricin-N-acetyltransferase (PAT) inhibiting glutamine synthetase, causing a fatal accumulation of ammonia.
On top of this transformation, line 1507 contains a second introduced gene, the truncated *Cry1F* gene from *Bacillus thuringiensis ssp aizawai*. This gene protects the maize against

[2] see: http://www.efsa.eu.int/science/gmo/gm_ff_applications/more_info/650_en.html

some Lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*.

b) The inbred line NK603 was developed to be resistant to the herbicide glyphosate by the introduction of a gene encoding a glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) from *Agrobacterium sp* strain CP4.

c) Both inbred lines were crossed; the resulting F1-hybrid 1507xNK603 contains the 3 genes.

d) Both inbred lines have been assessed previously.

Line 1507 was assessed for import and processing under Part C of Directive 2001/18/EC and also assessed for import, feed and industrial processing and cultivation under Part C of Directive 2001/18/EC and for food use under Regulation Nr. (EC) 1829/2003. It was approved for import, processing and feed uses under Directive 2001/18/EC by Commission Decission 2005/772/EC and for food uses under Regulation Nr. (EC) 1829/2003 by Commission Decision 2006/197/EC.

Line NK603 received EFSA opinions in favour of its authorization and was authorized under Directive 2001/18/EC by Commission Decision 2004/643/EC. The use for food and food ingredients was authorized under Regulation Nr. (EC) 258/97 by Commission Decision 2005/448/EC.

Scientific evaluation

The comments of experts of the Biosafety Advisory Council are summarized below.

1. The information on the number of inserts is adequately documented by multiple Southern blot analyses. Experiments were well performed and described with sound conclusions. The copy number of the transformations in the hybrid is identical to the copy number in the parental lines.

2. The nutrient composition of 1507xNK603 was extensively analysed and compared to non-GM-maize with a comparable genetic background. Replicated trials were conducted on different locations. For most components there were no significant differences between 1507xNK603 and the non-GM lines. **An expert indicated that the analysis of starch content is missing while it is the main carbohydrate component. The expert also considers the fibre analysis with the ADF and NDF method as not recommendable in food (these methods are standard methods to analyse fibre in forages and feed). He suggested to use total dietary fibre, both soluble and insoluble. EFSA had not made such comments.**

3. The transgenic proteins CRY1F, PAT and CP4 EPSPS occur throughout different parts of the hybrid plants. Protein concentrations are higher in forage than in kernels (Precise data can be read in the summary of the comments of the experts). The levels of expression of the 3 proteins in the hybrid do not differ significantly from their expression level in the parental lines. No unintended fusion proteins have been detected in the parental lines.

4. Maize food and feed and processed products derived from 1507xNK603 are equivalent to commercial maize and food, feed and processed products derive from commercial maize. Occasionally there were significant differences between the transgenic hybrid and the non-transgenic hybrid, but not in all locations. **Since high variabilities occur in the results as well as in useful reference literature data, one expert advised deeper research into the analysis of potential metabolites resulting from the inactivation and degradation of the herbicide and into the analysis of the newly expressed proteins. Although the protein concentrations are low, the expert thinks that it is possible that the concentration might increase in particular processed derivative products, owing to redistribution of particles during the processing. He advises a good post-marketing monitoring system in order to study further the potential concentration of secondary metabolites and anti-nutrients and allergens in cracked maize. He also wants to know if the defined daily consumption of 8,8 g maize per person comprises the processed ingredients used in a lot of products. In addition the statistical analysis of maize consumption is poorly documented.**

5. No toxicological problems are signalled.

6. In a well-documented broiler feeding study, it was concluded that 1507xNK603 maize is nutritionally equivalent to non-GM maize with comparable genetic background. An expert highly recommended **an additional 90-day rat feeding study to conform safety data from the broiler tests.**

7. The vertical potential for gene transfer is considered as unlikely owing to the absence of wild weedy relatives and of feral maize populations in Europe. Should kernels germinate, the probability of winter survival is unlikely due to their frost susceptibility.

8. A decline of non-target organisms resulting in a loss of biodiversity is considered as unlikely.

9. The general surveillance plan is vague and not detailed. An expert advises to improve this program should 1507xNK603 receive a positive advise.

Conclusion

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

Within the scope of the application, i.e. food or feed uses (excluding cultivation), the risks of 1507xNK603 hybrid maize might be low.

However a number of scientific arguments addressed in points 2, 4, 6, 9 (indicated in bold) here above plead for some extra research, improvement of the presented data or a better statistical underpinning of results.

p.o.

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

Annex I : Comments of experts in charge of evaluating application EFSA/GMO/UK/2004/05 (ref: BAC_2006_PT_356)

Afdeling Bioveiligheid en Biotechnologie /Section Biosécurité et Biotechnologie
Rue Juliette Wytsmanstraat, 14 - B 1050 Brussels - BELGIUM
Tel: 32-2-642.52.93 | Fax: 32-2-642.52.92 | Email: bac@sbb.ihe.be | Web server: http://www.bio-council.be/

Bioveiligheidsraad
Conseil de Biosécurité

28 April 2005

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2006/PT_356
Email. : bac@sbb.ihe.be

Expertise report for the EFSA dossier EFSA/GMO/UK/2004/05 - Compilation of all the comments received from the experts

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 12 december 2005
Coordinator: Prof. dr. ir. Dirk Reheul (UGent)
Experts: Yann Devos (UGent), Michel Paquot (FUSAGx), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: ecology, nature conservation, biodiversity, sustainable development, biosafety research, biochemistry of food/feed, industrial processing, novel food, genome analysis, genetic engineering, maize
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2004/05** concerns an application of the company **Pioneer Hi-Bred and Mycogen Seeds** for the marketing of the genetically modified **maize 1507 x NK603** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 1 April 2005.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

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

information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The product described in this application is 1507 x NK603 maize for all food and feed uses, and for all food, feed and processed products derived from 1507 x NK603 maize. The 1507 x NK603 maize has been obtained from traditional breeding methods between progeny of two genetically modified maize. The two GM maize are DAS-Ø15Ø7-1 maize, referred to as 1507 maize(expressing the CRY1F and PAT proteins) and MON-ØØ6Ø3-6 maize, referred to as NK603 maize (expressing the CP4 EPSPS protein). No new genetic modification has been introduced in 1507 x NK603 maize.

The CRY1F protein confers resistance to certain lepidopteron insect pests, such as the European corn borer and *Sesamia* spp. The PAT protein confers tolerance to glufosinate-ammonium herbicide and the CP4 EPSPS protein confers tolerance to glyphosate herbicide.

The types of products planned to be placed on the market according to the authorisation applied for include 1507 x NK603 maize for all food and feed uses, and for all food, feed and processed products derived from 1507 x NK603 maize in accordance with Regulation (EC) 1829/2003. In addition, this application requests authorisation for import and processing of 1507 x NK603 maize in accordance with Part C of Directive 2001/18/EC.

Comment 2

The intended use of the product is similar to other conventional maizes. By consequence, food used are concerned. Among these uses, the whole grains can be considered but also cracking of the grains in order to obtain mainly starch.
Native starch is a food ingredient but other important products are derivatives of starch : pregelified, modified or hydrolysed.
Following Syngenta, majority of maize is used for animal feeds, and about 8% of the grain is processed for human food products mainly by wet-milling or dry-milling. Nevertheless the food consumption of starch seems to be very high because starch (from maize, wheat,…) is a very important food ingredient. Maize grain is also processed into industrial products (11%), such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil. There are multiple categories of users of 1507 x NK603 maize, e.g. animal feed and milling industry, agriculture, skilled trades and consumer use by public at large.

Comment 3

The general information provided is sufficient / no questions.

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

Comments/Questions of the expert(s)

Information is detailed and sufficient / no questions.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Sufficient / no questions.

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)

Adequate and detailed description.

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

Comments/Questions of the expert(s)

The information on the number of inserts is adequately documented by multiple southern blot analyses. Experiments are well performed and described. Conclusions are sound.

Technical Question : In contrast with the extensive southern blot analyses (described in annex 7) to determine the copy number, it is not clear from the text (p13 Technical dossier) how the sequences at the site of the 1507 insert were obtained (sequence details provided in text and annex 8). Tail-pCR, Genome Walking, other method ?

Comment: In contrast to the complete information provided for the 1507 insert, the detailed information for NK603 is not provided. Instead there is a reference to an earlier notification and request for authorisation (p12 in summary document / p16 Technical dossier). Therefore it is not possible for me to make an assessment on the completeness of the molecular information for NK603.

D.3. Information on the expression of the insert

Comments/Questions of the expert(s)

Comment 1

Expression of CRY1F, PAT and CP4 EPSPS proteins in grain from 1507 x NK603 maize was characterized using a specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein.

The grain samples were taken from plots that were sprayed with glyphosate herbicide only; with glufosinate-ammonium herbicide only; and with glyphosate followed by glufosinate-ammonium herbicides.

Expression levels of the proteins CRY1F, PAT and CP4 EPSPS in grain from 1507 x NK603 maize were comparable regardless of the herbicide treatment. Expression of CRY1F protein in grain from 1507 x NK603 maize ranged from 0.53 to 2.43 ng/mg grain dry weight.

These results are comparable to the expression level of the CRY1F protein in grain from 1507 maize, which ranged from 1.2 to 3.1 ng CRY1F protein/mg tissue dry weight.

Expression of PAT protein in grain from 1507 x NK603 maize was below the lower limit of quantisation of the assay, which was 0.075 ng/mg grain dry weight. These results are comparable to the expression levels of the PAT protein in grain from 1507 maize, which were also below the lower limit of quantisation of the assay.

The mean expression level of CP4 EPSPS protein in grain from 1507 x NK603 maize was 7.18 ng/mg grain dry weight and ranged from 3.80 to 11.10 ng/mg grain dry weight. When expressed in terms of µg/g fresh weight, the mean expression level of CP4 EPSPS protein in grain from 1507 x NK603 maize was 6.24 µg/g fresh weight and ranged from 3.30 to 9.65 µg/g fresh weight. The levels of expression of the CP4 EPSPS protein in grain from 1507 x NK603 maize are comparable to the expression levels of the CP4 EPSPS protein in grain from NK603 maize, which ranged from 6.9 to 15.6 µg/g fresh weight. The notification of NK603 maize pursuant to Directive 2001/18/EC (C/ES/00/01) and the request for authorisation of NK603 maize pursuant to Regulation (EC) No. 258/97 submitted by Monsanto Europe S.A. have been authorised by Commission Decisions of 19 July 2004 and 26 October 2004, respectively.

The 1507 x NK603 maize expresses the proteins CRY1F, PAT and CP4 EPSPS throughout the different parts of the maize plant .

Southern analyses conducted on 1507 x NK603 maize have confirmed the molecular equivalence and identical copy number between the inserts found in 1507 x NK603 maize and those present in 1507 and NK603 maize, respectively.

A detailed evaluation of the absence of expression of potential fusion proteins in 1507 maize has been carried out by the EFSA GMO Panel (EFSA, 2004; Annex 4). In conclusion, no unintended CRY1F or PAT fusion proteins are expressed in 1507.

In addition, a detailed characterization of the absence of expression of potential CP4 EPSPS fusion proteins in NK603 maize has been included in the notification of NK603 maize pursuant to Directive 2001/18/EC (C/ES/00/01) and in the request for authorisation of NK603 maize pursuant to Regulation (EC) No. 258/97 submitted by Monsanto Europe S.A., which have been authorised by Commission Decisions of 19 July 2004 and 26 October 2004, respectively.

Comment 2.

All the products derived from the genetically modified maize may content the proteins CRY1F, PAT and CP4 EPS PS.
Even if the content is low (magnitude of 1ng, 10ng/mg grain dry weight), it is useful to check the risk of accumulation into specific derivatives products especially those obtained by cracking of the raw materials. In other word, can the content in CRY1F, PAT and CP4 EPS PS increase in a product of cracking for the food chain. Where does these proteins partition (starch fraction, oil,….)?

Comment 3

Sufficient information / no questions.

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

Comments/Questions of the expert(s)

Sufficient information / no Comments

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

Comments/Questions of the expert(s)

No comments / no questions

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

Comments/Questions of the expert(s)

Chances of transfer of genetic material to other organisms are negligible.

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)

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

The nutritional analysis was undertaken on a broad range of compounds in grain from 1507 x NK603 maize in accordance with OECD guidelines for assessment of GM maize. The objective was to determine that 1507 x NK603 maize treated with glyphosate, glufosinate-ammonium, or glyphosate followed by glufosinate-ammonium herbicides, was equivalent to non-GM maize with comparable genetic background.
Grain samples from 1507 x NK603 maize (all herbicide treatments) and non-GM maize with comparable genetic background were collected and analysed for nutrient composition, including: crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cysteine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine), minerals (phosphorus, calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc) vitamins (beta-carotene, vitamin B1, vitamin B2, folic acid, and vitamin E [alpha tocopherol isomer]), secondary metabolites (inositol, raffinose, furfural, p-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor).

In accordance with OECD guidelines substantial equivalence was evaluated by comparing the following: i) mean nutrient composition values of each 1507 x NK603 maize entry to non-GM maize with comparable genetic background and, ii) mean nutrient composition values of the 1507 x NK603 maize entry to nutrient ranges available in the published literature. Statistical analyses were conducted with data combined across all six locations as well as on a per location basis using data from the 3 replicates at each of the individual locations.

Annex 3 presents an important study entitled “Agronomic characteristics, quantitative ELISA and Nutrient Composition analysis of a maize Line Containing Events DAS-01507-1 and MON-00603-6 Derived from Conventional Crossings : Chile Locations

Annex 3 contains analyses reported to provide nutrient composition data for a substantial equivalency study.
Methods of analysis and representative calibration curves are presented.
Secondary metabolites and anti nutrients are also analysed.
The sampling method and the statistical analysis are explained.

Grain samples were collected at the same physiological maturity.
No statically significant differences in composition were generally observed across locations between 1507 x NK603 maize and non-GM maize treated with glyphosate and/or glufosinate. Some exceptions arose, for example, for crude fat, vitamin B1, manganese,…..depending also of the herbicide treatment.

In these cases, all mean values were within reported literature ranges where available. Nevertheless, a great variability exist in the reported literature, especially for crude protein, crude fat and carbohydrate. The analysis of the starch content is missing while it is the main carbohydrate component.

Results of proximate and fibre analysis do not allow to point out important differences between the mean values of the genetically modified maize and the control. Nevertheless, taking into account the standard error, some differences appears (eg. NDF content p79 annex 3, vitamin D1 p85 annex 3, vitamin E p85, 87 annex 3, ferulic acid p89 annex 3,…..)

Considering food application, the analysis of dietary fibre by acid detergent fibre (ADF) and neutral dietary fibre (NDF) is not recommended. The evaluation of total dietary fibre, soluble and insoluble one would be more obvious.

Dietary fibres may be composed of soluble and insoluble constituents. Because of the large diversity indigestible materials, analysis is difficult. Enzymatical methods are preferable to the Van Soest method even if this last technique has been standardized in some countries, especially for cereals.
The Van Soest method gives values similar to those obtained in vivo from digestibility studies with animals. This technique allows to determine the concentration of cellulose, lignin and hemicelluloses. Nevertheless, the Van Soest method does not correspond to the actual notion of dietary fibres including a lot of other constituents as soluble and insoluble fibres are not distinguished.
With enzymatical methods the digestible constituents (1-4 ß-glucans, proteins) in the defatted sample are enzymatically hydrolysed (heat stable α-amylase, gluco-amylase, protease). Water soluble fibres are isolated by precipitation with ethanol. The proteins and mineral matter still remaining with the soluble and insoluble dietary fibres are deducted.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

D.7.5 Product specification

Comments/Questions of the expert(s)

Following the requesting, the 1507x NK603 maize and all food, feed and processed products derived from 1507 x NK603 maize are substantially equivalent to commercial maize and all food, feed and processed products derived from commercial maize.
The detection is similar to the detection methods for 1507 and NK 603 maize.
Small significant differences are pointed out between 1507 x NK 603 maize and non-GM control maize. Generally, these significant differences were not observed for all individual locations.

Even if these differences are not numerous and important, a rigorous substantially equivalence can not be certified from the analysis. Comparison with reported literature ranges is not valid because of the too high reported variability.

- The presence of newly expressed proteins must be analysed (toxicology, partition during food processes,….see 7.8.1)
- As the transgenic plant inactivates the herbicide, metabolised products might be present in the plant.
  Information about this probability must be given for 1507 x NK603. Are there degradation products from the herbicides?

D.7.6 Effect of processing

Comments/Questions of the expert(s)

The production processes applied to maize are well known and have a long history of safe use.

1507 x NK603 maize will undergo existing production processes used for commercial maize. No novel production process is envisaged. In the EU, most of the maize is used for animal feed, and about 8% is processed into food products such as highly refined starch by the wet-milling process and maize flour by the dry-milling process. The majority of the starch is used for sweeteners and fermentation including high fructose maize syrup and ethanol. Starch is also a food ingredient. In addition to milling, the maize germ can be processed to obtain maize oil. These processed products of maize are used in a variety of food products.

The cracking of maize in order to produce different food ingredients especially starch, oils and proteins, may concentrate some secondary metabolites or anti nutrients and allergens (9 kd lipid transfer protein, 16 kd trypsin inhibitor,….).
Some of these compounds will be denatured during food processes especially by heat treatment. It is the same for the proteins CRY1F, PAT and CP4 EPSPS.
Nevertheless, the chemical composition of a raw material is not sufficient to predict the repartition of the minor components during a food process or the cracking.
The used technology is of course important but also the locations and the interactions between the different components in the raw materials.

Considering the difficulties to conduct such studies and, on the other hand, the existence of a risk, even weak, to concentrate a secondary metabolite in an other way than the traditional sources, recommendations of a post-market monitoring seem necessary.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

The 1507 x NK603 maize and all food, feed and processed products derived from 1507 x NK603 maize are expected to replace a portion of similar products from commercial maize with total consumption of maize products remaining unchanged. Therefore, the total anticipated intake/extent of use of maize and all food, feed and processed products derived from maize will remain the same.

The estimated consumption of maize by the European population is 8,8 g/person/day in the dossier. It is not specified if this estimation takes into account a lot of food products containing ingredients from cracking (starch especially, oils, ….). The consumption at the 95th percentile is not also specified. These estimations are necessary for the food application.

D.7.8 Toxicology

Comments/Questions of the expert(s)

The stacked 1507 x NK603 maize has been obtained from traditional breeding methods between two genetically modified maize. No new genetic modifications have been introduced in 1507 x NK603 maize. The parental lines contain the genes *cry1f*, *pat* (1507 maize), and *cp4 epsps* (NK603) conferring resistance to certain lepidopteran insects, and tolerance to herbicides containing the active ingredient glufosinate-ammonium or glyphosate. Both of the introduced traits from the parental lines are inherited in 1507 x NK603 maize. This results in the combined expression of the CRY1F, PAT, and CP4 EPSPS proteins in the same plant. NK603 has been approved for commercial import and processing in the EU (C/ES/00/01). EFSA issued positive assessments on 1507 maize. Eventually, the EU Commission approved the import of 1507 maize for use in animal feed (Nov 3, 2005) and food (March 6, 2006).

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

No new genetic modifications have been introduced in 1507 x NK603 maize.
The safety of the proteins CRY1F, PAT, and CP4 EPSPS has already been confirmed in detail in accordance with the applications for authorisation of NK603 and 1507 maize. A battery of tests designed to evaluate the CRY1F, PAT, and CP4 EPSPS proteins for characteristics associated with food allergens and toxins raised no concern. CRY1F, PAT, and CP4 EPSPS proteins shared no sequence homology with known toxins. There is a rapid digestion of the proteins in simulated digestive conditions, susceptibility to heating, and lack of acute toxicity for the CRY1F, PAT, and CP4 EPSPS proteins, as determined by the mouse acute oral toxicity study.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

See D.7.5 and D.7.6

Comment 2

No constituents other than the CRY1F, PAT, and CP4 EPSPS proteins are novel. 1507 x NK603 maize was shown compositionally equivalent to non-GM maize with comparable genetic background. Agreed.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

See D7.5 and D.7.6

Comment 2

No particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

The applicant concluded that the safety assessment for the individual proteins is not changed when combined in 1507 x NK603 maize obtained by traditional breeding methods.
A confirmatory animal feeding experiment was conducted using 1507 x NK603 maize fed to broiler chickens.

Poultry broiler feeding study.
A poultry feeding study was conducted over a 42-day periods with diets containing grains from 1507 x NK603 maize treated with glyphosate herbicide, treated with glufosinate-ammonium herbicide, or treated with glyphosate followed by glufosinate-ammonium herbicide. For comparison, diets containing grain from non-GM isoline maize and from three types of commercial maize were also fed to the chickens (Ross x Cobb, in total 840 animals, 10 broilers per pen with 12 pens per treatment). The animals were observed for overall health and behavioural changes. Body weights and feed weights were measured every 7 days. The body weight parameters evaluated at the end of the study

included carcass yield, thighs, breasts, wings, legs, abdominal fat, kidneys, and whole liver. Mortality, body weight and feed conversion of the chickens fed with the maize were compared. Meanwhile, all maize lines were evaluated for nutrient composition, expression levels of transgenic proteins, mycotoxins and pesticides. It was concluded that 1507 x NK603 maize is nutritionally equivalent to non-GM maize with comparable genetic background and to commercial maize, and that it is as safe as commercial maize.

According to the applicant, it is considered that it is scientifically valid to use data from the single GM lines 1507 and NK603 to support the safety assessment of the hybrid 1507 x NK603. Well-performed field experiments with agronomic data and a compositional study, and a broiler chicken feeding study performed with 1507 x NK603 maize, support the conclusion that adverse effects are highly unlikely to occur following oral exposure to 1507 x NK603 maize. We can agree for most of the reasoning.
Although broiler chickens are the livestock animal of choice for confirming nutritional equivalence, confirmatory data for the safety assessment of the hybrid 1507 x NK603 is needed, in particular, the need for an additional 90-day rat feeding study, including complete endpoints (biochemical, haematological, histological), with the hybrid 1507 x NK603 to exclude any adverse effect on human health. In this rodent feeding study, experimental treatments should include the GM crop, a non-GM counterpart with comparable genetic background, and a range of commercial non-GM controls. As in the already performed studies the diets should contain grain from 1507 x NK603 maize treated with glyphosate herbicide, treated with glufosinate-ammonium herbicide, or treated with glyphosate followed by glufosinate-ammonium herbicide.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

The key nutrients have been analysed except the carbohydrates fraction (simple and complex), starch and dietary fibre.

See also D.7.3.

The carbohydrates fraction is obtained by difference and not characterized excepted for raffinose. Dietary fibre is estimated via ADF and NDF, not relevant for food use.

Potential toxicants and anti-nutrients have been measured but no information is given about the effect of processing (derived food products) upon the content and distribution of nutrients and anti-nutrients considering that a substantial equivalency with traditional maize is argued.

Even if the genetic modification does not change significantly the overall nutrient, investigations to what extent the different industrial processes can lead to the concentration or the elimination of the

minor constituents are useful. Otherwise a surveillance programme should accompany the marketing of derived products from 1507x NK603 maize.

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

Comments/Questions of the expert(s)

No risks to human health have been identified by comparison of commercial maize. The substantial equivalency of 1507 x NK603 maize and commercial maize is argued.
It should be recommended to have a post-market monitoring taking into account previous comments.

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

Comments/Questions of the expert(s)

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)

- See 9.3.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

- See 9.3.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

- Establishment of invasive feral 1507 x NK603 maize populations (vertical gene flow): unlikely.
During transport of the imported maize F_2 grains some grains will inevitably be spilled accidentally. The probability that these spilled grains result in self-sustaining and/or invasive feral maize populations is *nihil*. Maize has been grown for many years in the EU and feral maize populations are rarely observed. Even in agricultural fields with ideal conditions, maize is not known to persist as a weed: volunteers are killed by frost. In Mediterranean countries, some volunteers occur but these are

easily controlled by current agronomic practices. Due to its high level of domestication, the maize germination and establishment potential outside the fields is expected to remain very low even despite the acquisition of the new transgenic traits (that confer a selective advantage under field conditions with the appropriate selection pressure). It is important to bear in mind that the imported grains that can be spilled are from the F_2 generations and that these are expected to be less fit than F_1 grains.

- Acquisition of transgenic traits by wild/weedy maize relatives resulting in invasive progeny (vertical gene flow): unlikely.
There are no wild/weedy relatives of maize in the EU. However, cultivated maize occurring in the neighbourhood of the feral 1507 x NK603 maize could theoretically act as a recipient. This hypothesis seems not very probable. First, feral maize populations resulting from spilled grains are rarely observed and it is even more doubtful that these plants will flower. Second, pollen flow would only occur locally. The pollen amount will be small compared to a field of commercial size. As maize pollen is heavy, pollen will be deposited within a few meters of the source. Third, it is not clear that feral maize plants will flower and release their pollen at the moment of silking of the recipients.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

- Corn rootworm develops resistance requiring alternative pest control: unlikely.
- Other pests take become abundant due to the efficient control of ECB/MCB: unlikely.
The number of feral 1507 x NK603 plants will be extremely low and the selective pressure against the ECB/MCB small.

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

Comments/Questions of the expert(s)

- Decline of non-target organisms resulting in the loss of biodiversity and/or ecological functions: unlikely.
The number of feral 1507 x NK603 plants will be small as a result of what the environmental exposure of 1507 x NK603 will be extremely low.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

- Accidental consumption of maize plant parts leading to toxicological and/or allergenic reactions: unlikely.
See food and feed experts.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

- Accidental consumption of maize plant parts leading to toxicological and/or allergenic reactions: unlikely.
See food and feed experts.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

- Decline of non-target organisms resulting in the loss of biodiversity and/or ecological functions: unlikely.
- Impact on carbon and nitrogen recycling through changes in soil decomposition of organic material: unlikely.
The exposure to spilled 1507 x NK603 grains and feral 1507 x NK603 plants will be extremely low as a result of what the environmental exposure to toxins produced by 1507 x NK603 (*e.g.* through root exudates and decaying plant parts) will be extremely low.

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

Comments/Questions of the expert(s)

- Not of application.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

The general surveillance (GS) remains very vague: a general frame of principles is not even provided. It might be advisable to complete the GS with more details once a positive advice of EFSA is obtained (*e.g.* include the names and coordinates of the networks that will be involved in the GS, provide the countries/regions where the GS will be done, give the information asked in the questionnaires).

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

There is no need to foresee a case-specific monitoring (CSM), since the risks were extremely low and no uncertainties remained in the ERA. CSM is not mandatory but required when uncertainty remains.

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

Comments/Questions of the expert(s)

On the opposite of the CSM, GS is mandatory for viable GM material. Non-viable GM material falls beyond the scope of the environmental monitoring provision. In case of imported viable grains, GS should consider that if substantial loss, spillage and establishment are possible, appropriate management systems should be in place to restrict environmental exposure. Given the increasing number of notifications covering import, unforeseen (cumulative) effects should be followed up in the GS.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

Eastham K, Sweet J (2002) Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Environmental Issue Report 28, EEA, http://reports.eea.eu.int/environmental_issue_report_2002_28/en/GMOs%20for%20www.pdf