**Bioveiligheidsraad**
**Conseil de Biosécurité**

***08-09-2015***

**Secretariaat**
**Secrétariat**

O./ref.: WIV-ISP/41/BAC/2015_0596

**Title:** Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/NL/2010/80 from Monsanto under Regulation (EC) No. 1829/2003

## Context

The application EFSA/GMO/NL/2010/80 was submitted by Monsanto on 21 May 2010 within the framework of Regulation (EC) No. 1829/2003[1] for authorisation of genetically modified (GM) maize NK603 x T25 for food and feed uses, import and processing.

Maize NK603 x T25 was obtained by conventional crossing (no new genetic modification involved) of two GM lines containing the following single events:
- Line NK603 expressing the CP4 EPSPS and CP4 EPSPS l214p (CP4 5-enolpyruvylshikimate-3-phosphate synthase) proteins conferring tolerance to glyphosate (N-(phosphonomethyl)glycine)-based herbicides, and
- Line T25 expressing the PAT (phosphinothricin acetyl transferase) protein conferring tolerance to glufosinate ammonium-based herbicides.

The application was officially acknowledged by EFSA on 12 October 2010. 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 GM 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). Five 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 the list of comments actually placed on the EFSAnet on 12 January 2011.

The opinion of the EFSA GMO Panel was adopted on 24 June 2015 and published on 15 July 2015 (EFSA Journal 2015; 13(7):4165[2]). The responses from the Panel to comments submitted by the experts during the three-month consultation period were made available on 16 July 2015.

On 22 July 2015 the EFSA opinion and the responses from the EFSA GMO Panel were forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the 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.

[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/4165.htm

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The comments formulated by the experts together with the EFSA opinion including the answers of the EFSA GMO Panel, form the basis of the advice of the Biosafety Advisory Council given below.

It should be also noted that the two single maize events NK603[3] and T25[4] have already been assessed positively (no risk identified to human and animal health or to the European environment) by the Belgian Biosafety Advisory Council, and that maize NK603 and T25 are both already authorised in the EU for food and feed uses with the exception of GMO cultivation[5].

## Scientific evaluation

### 1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment[6].

### 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 notes that the experimental design used for the comparative analysis was not fully optimal. Data for compositional analysis should include information on both maize NK603 × T25 sprayed with target herbicides and maize NK603 × T25 not sprayed with target herbicides. This latter set of information was missing for the compositional analysis, whereas it was available for the analyses of agronomic and phenotypic characteristics.
Nevertheless, the Council agrees with EFSA that the comprehensive set of data available on the composition and agronomic/phenotypic characteristics (including the fact that both single events do not raise concern) allows concluding that the compositional analysis of GM maize NK603 x T25 does not raise safety concerns.

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 Biosafety Advisory Council has evaluated the safety of the newly expressed CP4 EPSPS, CP4 EPSPS l214p and PAT proteins in the context of previous applications, and no concerns were identified. Taking into account the updated information provided by the applicant, the Council is of the opinion that this conclusion remains valid.

[3] Advice of the Belgian Biosafety Advisory Council of 2 October 2009 on applications EFSA/GMO/NL/2005/22 and EFSA/GMO/RX/NK603 (ref WIV-ISP/BAC/2009_01367)
[4] Advice of the Belgian Biosafety Advisory Council of 21 May 2014 on applications EFSA/GMO/NL/2007/46 and EFSA/GMO/RX/T25 (ref WIV-ISP/41/BAC/2014_0329)
[5] EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=61 for NK603 and http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=20 for T25
[6] Since this application does not imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event should not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed CP4 EPSPS, CP4 EPSPS l214p and PAT proteins in the context of previous applications, and no concerns were identified.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity.
With regard to the allergenicity of the whole GM plant, maize is not considered to be a common allergenic food. Based on the available information, the Biosafety Advisory Council considers that there is no evidence that the overall allergenicity of maize NK603 x 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 to conclude that the nutritional characteristics of maize NK603 × T25-derived food and feed are not expected to differ from those of 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 NK603 x 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.

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

*Annex I : Compilation of comments of experts in charge of evaluating application EFSA/GMO/NL/2010/80 and comments submitted on the EFSAnet (ref. BAC_2011_0037)*

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***12-01-2011***

**Secretariaat**
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**Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2010/80**
**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 25 October 2010
**Coordinator:** Prof. Dirk Reheul
**Experts:** Leo Fiems (ILVO), Johan Grooten (UGent), Peter Smet (Consultant), Wim Stevens (UIA), Bart Van Droogenbroeck (ILVO).
**Domains of expertise of experts involved:** Molecular characterisation, agronomy, breeding techniques, animal nutrition, toxicology in vitro, general biochemistry, allergology, ecology, plant biology, risk analysis, maize.
**Secretariat (SBB):** Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

## INTRODUCTION

Dossier **EFSA/GMO/NL/2010/80** concerns an application of the company **Monsanto** for the marketing of the genetically modified **maize NK603 x T25** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 12 October 2010.
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). 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.
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**

## GENERAL COMMENTS

Comments/Questions of the expert(s)

*Comment 1*

- Genetically modified maize NK603 × T25 is obtained by traditional breeding of two genetically modified maize lines, and no new genetic modification has been introduced
- NK603 maize was considered as safe as conventional maize; therefore, it could be placed on the market for food or feed or processing without an adverse effect on human or animal health or on the environment (EFSA, 2009)
- T25 maize has been temporarily approved by the European Commission to place on the market (Anonymous, 1998).
- 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.

These features may be an advantage with regard to the evaluation of the application of NK603 × T25 maize.
Several investigations with genetically modified crops with stacked traits showed no deleterious effect. Therefore, it can be assumed that genetically modified maize NK603 × T25 is safe for animal and human health.

## A. GENERAL INFORMATION

Comments/Questions of the expert(s)

*Comment 1*

Technical Dossier, Part I, pg. 14. "*The ability to utilize glyphosate and glufosinate-ammonium in maize production offers farmers additional flexibility for broad-spectrum weed control.*" It could be of interest to be more descriptive and illustrate the value of the combination of the two HR traits in more detail, i.e. from a more practical point of view. If this is discussed in more detail further in the dossier, referring to the relevant section would make the dossier more comprehensive, easier to read.

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

Comments/Questions of the expert(s)

## C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

*Comment 1*

No comments for this section as the stacked event in this dossier was produced by conventional crossing. The authors further refer to the detailed information related to the genetic modification for the two authorized single events that were used to establish the stacked event under investigation.

Technical Dossier, Part I, pg. 24, Table 3 - "*TS-CTP2 - DNA sequence for chloroplast transit peptide, isolated from Arabidopsis thaliana EPSPS, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee et al., 1987)*". Is aromatic AA synthesis a prerequisite for a functional EPSPS? If so, it could be of interest to formulate this statement more explicit and clear for reasons of clarity.

Technical Dossier, Part I, pg. 25, Table 4 – In table 4 for the P35S promoter it is mentioned as "*high level constitutive expression promoter*". This descriptive information is not given for the same promoter and the rice actin promoter in Table 3 – of interest for reasons of uniformity.

## 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*

Technical Dossier, Part I, pg. 26, Fig. 1 – In the schematical representation of the *Msc* I/*Sca* I generated restriction fragments the larger fragment fo 6.1 kb is not delimited at the left side by a short vertical bar. I suggest to do so to discriminate this fragment from other fragments with an unknown size as e.g. the *Nco* I restriction fragments depicted in the same figure.

Technical Dossier, Part I, pg. 27, Fig. 2 – In the legend of this figure is stated "*Probe 2 was used in the Southern analysis and its location is depicted on the map*" The authors refer to the probe 2 described in Fig. 1 but do not mention this explicitly. It would be good to do so.

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

Comments/Questions of the expert(s)

*Comment 1*

Technical Dossier, Part I, pg. 29 – Conclusion. This paragraph ends by the sentence: "*NK603 × T25 therefore contains:"* without further text. On the following two pages two figures are given, the next page with text starts with ***"(b) The organization of the inserted genetic material at the insertion site and methods used for characterization"*** – indicating that some text is missing here. Please complete or omit last sentence from this paragraph.

Technical Dossier, Part I, pg.32 – second paragraph in section D1 (b) - Based on the text in this section and footnotes 14 and 15, the reader could conclude that 2 different bioinformatics evaluations (one for NK603 and one for T25) were performed to determine if any coding sequences were disrupted by the insertion of DNA in or whether coding sequences from the maize genome are present in the genomic DNA adjacent to the inserted DNA. Different databases were used as well. Confusion could be avoided by following the same approach for both events. In addition nothing is mentioned about an analysis to evaluate the presence and functionality of possible novel chimaeric ORF, as requested by "Guidelines for Molecular Characterization of Genetically Modified Higher Plants to be Placed on the Market" from WIV-SBB, Final version Feb 18, 2003.

Technical Dossier, Part I, pg.32 – (c) It is not explicitly mentioned that the 2 deletions detected did not affect any gene function.

## D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

*Comment 1*

Technical Dossier, Part I, pg.33. "*These field sites were representative of maize producing regions suitable for commercial production*." This is a very general statement. USA or European regions? Both? No reference climatic conditions are given, allowing comparison for the reader. A more detailed description of the trial sites from which samples were connected would be of interest.

Technical Dossier, Part I, pg.38 - **(c) Expression of potential fusion proteins** - Based on the text in this section and footnotes 17 and 18, the reader could conclude that 2 different bioinformatics evaluations (one for NK603 and one for T25) were performed to determine if any potential fusion protein was expressed. It seems logical to follow the same analysis for both insertion sites.

## 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 comments as the data provided illustrates that no significant differences where found between GM plant and recipient plant for all characters evaluated.

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

Comments/Questions of the expert(s)

*Comment 1*

The genetic stability of the inserts in NK603 x T25 hybrid and its progeny is discussed only very briefly. Though highly unlikely, nothing is said about potential 1) recombination following translocation process between the sequences that are homologous in the two constructs (e.g. p35S) and 2) silencing. These aspects disserve at least some attention in the discussion to confirm that they are highly unlikely to occur.

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

Comments/Questions of the expert(s)

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

### D.7.1 Comparative assessment

Comments/Questions of the expert(s)

*Comment 1*

The recommended antinutricients were controlled for. Although for raffinose a significant difference between NK603 × T25 and its control occurs, test values are within the 99% tolerance interval determined from commercial conventional hybrids grown at the same locations and time as the test maize.

### D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

*Comment from the SBB*

*Concerning the production of material for comparative assessment, good practices should be that field trials include blocks of GM plants exposed to the intended herbicide, blocks of GM plants not exposed to the herbicide and blocks of control plants not exposed to the herbicide. « Not exposed to the herbicide » means in that case not exposed to the herbicide to which the GM is tolerant but exposed to herbicides used in conventional treatments. In the absence of any weed control, both yield, composition and quality would be indeed negatively affected.*

*The current application is not fully clear as regards the agronomic conditions under which plants (GM or non GM) not exposed to glyphosate/glufosinate have been grown.*

*On the one hand, it is mentioned (Alba, 2009) that « NK603 × T25 plants were grown in the presence of herbicide treatment (post-emergence treatment with glyphosate and glufosinate). The conventional control substance was grown in the absence of herbicide treatment ».*

*On the other hand, on page 74 of the technical dossier, it is stated that « All the plants were grown under normal agronomic field conditions for their respective geographic regions. »*

*Can we assume that « under normal agronomic conditions » means « treated with conventional herbicides » ?*

### D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

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### D.7.4 Agronomic traits

Comments/Questions of the expert(s)

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### D.7.5 Product specification

Comments/Questions of the expert(s)

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### D.7.6 Effect of processing

Comments/Questions of the expert(s)

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### D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

### D.7.8 Toxicology

Comments/Questions of the expert(s)

*Comment 1*

The amount of PAT protein present in NK603 x T25 maize grain is small: 0.59 µg/g dry matter. 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).
Several studies conducted with feeds containing modified maize, expressing the CP4 EPSPS or the PAT protein, did not show any deleterious effect on animal health (see 9.7. for further comments).

*Comment 2*

Concentrations of CP4 EPSPS and PAT proteins in NK603 × T25 are comparable tot those in NK603 and T25 respectively.

### D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

*Comment 1*

a) Degradation of the CP4 EPSPS protein in simulated gastric fluid (dossier 22).
CP4 EPSPS was shown to be rapidly degraded in both an *in vitro* simulated gastric fluid (SGF) digestion model and an *in vitro* simulated intestinal fluid (SIF) model (Leach *et al.*, 2002; Ream *et al.*, 1993).

b) Degradation of the CP4 EPSPS protein in simulated intestinal fluid (dossier 22).
See above.

c) CP4 EPSPS: Acute Oral Toxicity Study in Mice (dossier 22).
There were no treatment-related adverse effects in mice administered the CP4 EPSPS protein by oral gavage at dosages up to **572 mg/kg**.

d) CP4 EPSPS: Assessment of Amino Acid Sequence Homology with Known Toxins (**From CBI**: Tu and Silvanovich, 2010b)
The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the CP4 EPSPS protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.

e) Degradation of the PAT protein in simulated gastric fluid (dossier 46).

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.

f) Degradation of the PAT protein in simulated intestinal fluid (dossier 46).
Furthermore, results obtained with a similar method coupled with a Western blot, under GLP conditions, show the almost immediate degradation of the PAT protein in simulated intestinal fluids (SIF) (pH 7.5), in the presence of pancreatin.

g) PAT: Acute Oral Toxicity Study in Mice (dossier 46).
The results showed that the animals treated with the PAT protein at **10 mg/kg** had no visible signs of systemic toxicity (intravenous route).

h) PAT: Repeated dose oral toxicity (14-day feeding) study in rats (dossier 46).
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.
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.

h) PAT: Assessment of Amino Acid Sequence Homology with Known Toxins (**From CBI:** Ranjan, 2010b)
The PAT protein from *pat* gene shows a high degree of homology with other proteins of its respective family. No records were found on potential hazard associated with this protein family.
Therefore, the PAT protein does not evidence any potential for inherent toxic properties.

### D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

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### D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

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### D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

*Comment 1*

a) 42-day feeding study in broiler chickens (**From CBI:** CQR-09-010, 2010).
There were no biologically relevant differences in broiler performance, carcass yield or meat composition between broilers fed diets containing NK603 × T25 corn and those fed diets produced from conventional control corn.

b) 90-Day rat feeding study ().
No further testing is needed.

### D.7.9 Allergenicity

Comments/Questions of the expert(s)

*Comment 1*

Although the allergenicity of the newly expressed proteins has been assessed following the criteria of the Codex Alimentarius 2003 and the proteins fullfill for the 4 criteria of de codex (1. Protein of non-allergenic source, 2. protein represents only a very small portion of the total protein, 3. Protein does not share structural similarities to known allergens, 4. Protein is rapidly digested in simulated digestive fluids), some concern still remains:

1. Allergy to maize has been reported see Maniu et al (2010), Krishnan et al (2010), Pastorello et al (2009), Fasoli et al (2009), Scibilia et al (2008).

In addition some remarks can be made (Ebo and Stevens, 2001)

2. Very low doses of plant proteins/allergens can induce allergic reactions (eg peanut allergen, nut allergen, …)
3. The introduction of new proteins or peptides can alter the three dimensional structure of a protein, exposing in that way new conformational parts of the protein, which can become allergenic
4. The rapid digestibility of a protein does not warrant non-allergenicity; some labile proteins are allergenic (eg Mal d 1 from apple)

*Comment 2*

A rapid degradation of the PAT or the CP4 EPSPS protein occurred in simulated gastric fluid (SGF); however, a rapid in vitro digestion is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005). 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. However, the combination of several analyses, resulting in a holistic, integrative approach, means that the chance for allergenic reactions may be very low.

*Summary of comments 1 and 2 rephrased by the coordinator*

Although the allergenicity of the newly expressed proteins has been assessed following the criteria of the Codex Alimentarius 2003 and the proteins fullfill for the 4 criteria of de codex, post-market monitoring seems mandatory, since e.g. a rapid in vitro digestion is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005), since the use of the SGF technique to predict the allergenic status of the proteins remains uncertain (Bannon et al., 2003; Herman et al. 2006) and since the introduction of new proteins or peptides can alter the three dimensional structure of a protein, exposing in that way new conformational parts of the protein, which can become allergenic.
A possible future application could be the introduction of component resolved diagnosis (De Knop et al, 2010).

*Comment 3*

Both heterologous proteins, CP4 EPSPS and PAT, expressed in NK603 × T25 maize GM plant have been the subject of previous separate evaluations. In these previous dossiers a weight-of-evidence analysis was performed with regard to allergenic potential of the respective proteins. These analyses were performed according to the recommendations by the Codex Alimentarius Commission, 2003 and comply in great lines with the recommendations made by the EFSA GMO panel scientific opinion on the assessment of allergenicity of GM plants (EFSA Journal 2010; 8(7): 1700).
- With regard to new expression of CP4 EPSPS, the allergenic risk was previously evaluated by undersigned as “The analysis of multiple parameters associated with or indicative of allergenic potential does not indicate individually or combined an increased risk for allergenicity”. - Also the second newly expressed protein, PAT, was part of a previous evaluation by the EFSA (EFSA-GMO-NL-2007-46 (Bayer CropScience, 2007). This analysis encompassed a sequence homology comparison to known allergens and a protein (functional) stability analysis under acidic and simulated gastric and intestinal conditions. In so far the previous EFSA reviewer(s) of this dossier did not suggest an increased risk of allergenicity, the lack of sequence homology with known allergens deduced from the in silico analysis and the sensitivity of the PAT protein to degradation under conditions mimicking oral uptake minimize the likelihood that the PAT protein upon consumption will elicit an allergenic reaction.
- Additional arguments counter indicative for an increased allergenic risk are the non-allergenic sources of both proteins and the relatively low presence of the proteins, expressed as fraction of total protein, in the seed of NK603 × T25.

**Conclusion.**
The above weight-of-evidence analysis does not indicate an increased risk for allergenicity of the NK603 × T25 maize GM plant.

### D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

*Comment 1*

Section 7.6. of the dossier does not include dried distillers grains and solubles (DDGS) as a co-product of the bio-ethanol production from maize. Its importance may increase in the coming decades.

Even if Europe is more dependent on wheat for the production of biofuels, maize grain is often mixed with wheat for biofuel production. The protein content in DDGS is approximately three times as high as in maize grain. The same remark is made with regard to section 7.7a. This also means that the concentration of PAT and CP4 EPSPS proteins may be three times as high as in maize grain. DDGS is an interesting feed, for monogastric animals as well as for ruminants, because of its high protein content.

Tables 10-14 of the Technical dossier only give an idea about the chemical composition, but digestibility is ignored. As there is no information about digestibility, it is not proven that GMO-maize is as nutritious as non-GMO maize. However, several animal feeding experiments demonstrated that both GMO and non-GMO maize crops are equivalent with regard to their nutritional value. It is recommended to add digestibility as a parameter to assess the feed quality.
*(last sentence added by the coordinator)*

The different fat content reported for birds receiving diets containing the conventional control or GM maize (P.101 of the Technical dossier) was not confirmed by McNaughton et al. (2007), where broiler chickens were fed genetically modified maize containing cry34Ab1 and cry35Ab1 genes and the pat gene.

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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## 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)

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### D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

### D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

### D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

### D.9.6 Effects on human health

Comments/Questions of the expert(s)

*Comment 1*

The digestive process of the laying hen effectively breaks down the CP4 EPSPS protein from soybean meal (Ash et al., 2003). Consequently, no modified protein is expected to be found in the egg, so that a detrimental effect on human health is nearly excluded. There is no reason to assume that the CP4 EPSPS protein from maize NK603 x T25 will not behave similarly.

### D.9.7 Effects on animal health

Comments/Questions of the expert(s)

*Comment 1*

Hammond et al. (2004) reported that the introduction of CP4 EPSPS protein into the corn genome is safe for human and animal health. Similarly, Hérouet et al. (2005) reported no harm from the inclusion of the PAT proteins in human food or in animal feed.

Several experiments have been conducted with genetically modified crops with stacked traits, involving different types of animals. No detrimental effects of the pat gene alone have been reported in poultry fed soybean meal (Ash et al., 2003), or of the pat gene in combination with other inserted genes in dairy cows fed maize silage (Phipps et al., 2005; Faust et al. (2007), in finishing beef cattle fed maize grain (Sindt et al., 2007; Huls et al., 2008), in laying hens fed maize grain (Schiedeler et al., 2008) and in growing-finishing pigs fed maize grain (Stein et al., 2009).

#### D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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### D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

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### D.11. ENVIRONMENTAL MONITORING PLAN

#### D.11.1 General

Comments/Questions of the expert(s)

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#### D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

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#### D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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### D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

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