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

***17-12-2010***

**Secretariaat**
**Secrétariat**

O./ref.: WIV-ISP/41/BAC/2010_1160[1]

**Title:** Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2009/65 from Dow AgroSciences and Monsanto under Regulation (EC) No. 1829/2003

## Context

The application EFSA/GMO/NL/2009/65 was submitted by Dow AgroSciences and Monsanto on 6 February 2009 for the marketing (import and processing) of the insect resistant and glyphosate/glufosinate-tolerant genetically modified MON89034 x 1507 x NK603 maize for food and feed uses under Regulation (EC) No. 1829/2003[2].

The application was officially acknowledged by EFSA on 5 August 2009. 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 (GMOs) 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 Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB). Seven 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 5 November 2009.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 8 September 2010 (The EFSA Journal, 2010, 8 (9):1782)[3], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 29 September 2010 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. In addition, the complementary information sent by the company to EFSA after 6 November 2009 was provided to the coordinator and to the sole experts who evaluated the compositional analysis and the toxicological aspects of this GM maize.

[1] Revised version completed with minority declaration
[2] 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)
[3] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1782.htm

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The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.
In addition, the scientific evaluations of the single events, namely maize line MON89034 (EFSA/GMO/NL/2007/37), maize line 1507 (EFSA/GMO/NL/2004/02) and EFSA/GMO/RX-1507) are taken into account in this advice. The Biosafety Council formulated a positive advice for line MON89034. but. For line 1507, due to the lack of quality of animal trials for toxicity testing and testing of the nutritional value provided by the applicant the Biosafety Advisory Council not to draw conclusions about the feed safety of this GM maize[4].
The three single maize events are authorised by the European Commission for food and feed uses[5].

## Scientific evaluation

### 1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the 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
With regard to compositional analysis, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.2. Assessment of toxicity
With regard to toxicity, 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
Maize is not a major allergen source. The potential allergenicity of the newly introduced proteins has been assessed. No allergenicity assessment was performed on the whole GM maize. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.
In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM plant in monitoring systems.

[4] Advice of BAC on maize line MON89034: BAC_2009_0880; Advice of BAC on maize line 1507: BAC_2009_01368; Advice of BAC on maize line MON88017: BAC_2009_01045; Advice of BAC on maize line 59122: BAC_2007_SC_536;
[5] See GMO register : < http://ec.europa.eu/food/dyna/gm_register/index_en.cfm>
[6] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

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

As the allergenicity of the whole GM maize has not been 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 EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

Agrees with the GMO panel of EFSA that

"maize MON89034 x 1507 x NK603 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses".

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

p.o. Dr. Philippe Herman
Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

*Annex I : Minority declaration*
*Annex II: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2009/65 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2009_01479)*

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

*05-11-2009*

**Secretariaat**
**Secrétariat**

N./réf. : WIV-ISP/15/BAC_2009_01479
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**Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2009/65**
**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 31 August 2009
**Coordinator:** Françoise Vancutsem
**Experts:** Pascal Cadot (Consultant), Eddy Decuypere (KUL), Rony Geers (KUL), André Huyghebaert (UGent), Peter Smet (Consultant), Jan Van Doorsselaere (KH Zuid-West Vlaanderen), Hadewijch Vanhooren (KUL)
**Domains of expertise of experts involved:** Genetics, molecular characterisation, human nutrition, animal nutrition, analysis food/feed, substantial equivalence, traceability of alimentary chain, toxicology in vitro and in vivo, general biochemistry, immunology, alimentary allergology, ecotoxicology, herbicide tolerance
**Secretariat (SBB):** Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

## INTRODUCTION

Dossier **EFSA/GMO/NL/2009/65** concerns an application of the company **Dow AgroSciences & Monsanto** for the marketing of the genetically modified **MON89034 x 1507 x NK603 maize** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 05 August 2009.
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*

Mon89034 produces Cry1A.105 and Cry2Ab2.
Mon1507 produces Cry1F and PAT (phosphino-thricin-N-acetyltransferase) providing tolerance to glufosinate-ammonium.
NK603 produces CP4 EPSPS, a glyphosate tolerant 5 enolpyruvyl-shikimate-3 phosphate synthase.
Traditional breeding methods are used to combine the 3 genetically modified maize lines into Mon89034 x 1507 x NK603 and no new genetic modification has been introduced.

### A. GENERAL INFORMATION

Comments/Questions of the expert(s)

*Comment 1*

No comments.

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

Comments/Questions of the expert(s)

*Comment 1*

No comments

*Comment 2*

No comments.

### C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

No comments.

## D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

No comments.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

No comments.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

Cry1A.105 is present in grain of MON89034 at 1.7-3.5 ng/mg dry weight which is lower than the range of 3.4-5.8 ng/mg dry weight in MON89034x1507xNK603. This was also shown in table 5 (p 86), but no explanation why this is found was given.

Cry1F is present in comparable levels in Mon1507 and MON89034x1507xNK603.

Cry2Ab2 is present in comparable levels in Mon89034 and MON89034x1507xNK603.

CP4 EPSPS is present in comparable levels in NK603 and MON89034x1507xNK603.

*Comment 2*

No comments.

**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 differences in agricultural characteristics as for reproduction, dissemination, survivability or other characteristics were observed.

*Comment 2*

No comments.

**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 comments.

**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*

Almost nonexisting possibilities for transfer of genetic material to other plants in case of unintended release of MON89034x1507xNK603 maize e.g. via spillage during transportation of grain since the scope of this application does not include authorization for the cultivation of MON89034x1507xNK603 maize seeds products in EU.

*Comment 2*

No comments.

## 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*

Both commercial maize (14 conventional hybrids) as well as a comparative assessment with non-GM control maize with comparable genetic background as the MON89034x1507xNK603 maize has been used as the baseline.

*Comment 2*

Analytes determined in grain:

| **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) | X | phosphorus | X |
| neutral detergent fiber (NDF) | X | potassium | X |
| total detergent fiber (TDF) | X | selenium | |
| starch | | sodium | |
| | | zinc | X |
| | | total nitrogen | |

| Vitamins | | Amino acids | | Fatty acids | | Secondary metabolites | | Antinutrients | |
|---|---|---|---|---|---|---|---|---|---|
| A (β-carotene) | X | alanine | X | 14:0 myristic | | ferulic acid | X | phytic acid | X |
| B1 (thiamine) | X | arginine | X | 15:0 pentadecanoic | | | | Stachyose | |
| B2 (riboflavin) | X | Asparagine | | 16:0 palmitic | X | furfural | | raffinose | X |
| B3 (niacin) | X | aspartic acid | X | 16:1 palmitoleic | | inositol | | trypsin inhibitor | |
| B6 (pyridoxine) | X | Cysteine | X | 18:0 stearic | X | *p*-coumaric acid | X | Gossypol | |
| B9 (folic acid) | X | glutamic acid | X | 18:1 oleic | X | | | malvalic acid | |
| C (ascorbic acid) | | Glycine | X | 18:2 linoleic | X | | | sterculic acid | |
| E (α-tocopherol) | X | Histidine | X | 18:3 linolenic | X | | | dihydrosterculic acid | |
| | | Isoleucine | X | 20:0 arachidic | X | | | | |
| | | Leucine | X | 20:1 gadoleic | X | | | | |
| | | Lysine | X | 22:0 behenic | X | | | | |
| | | Methionine | X | 24:0 lignoceric | | | | | |
| | | phenylalanine | X | | | | | | |
| | | Proline | X | | | | | | |
| | | Serine | X | | | | | | |
| | | Threonine | X | | | | | | |
| | | Tryptophan | X | | | | | | |
| | | Tyrosine | X | | | | | | |
| | | Valine | X | | | | | | |

Conclusion:
Statistic significant differences between MON 89034 × 1507 × NK603 and XE6001 (same genetic background) occur, but the mean value is always within the range provided by the commercial reference lines. The only exception is copper in grain. The mean value for MON 89034 × 1507 × NK603 lies not within this range but still falls in the range found in the literature.

*Comment 3*

Maize MON89034 x 1507 x NK603, submitted in the application, will be further described as maize 65. Maize 65 is compared with a conventional maize with similar genetic background and with commercial maize hybrids.
This is an accepted approach for this part of the application, as it was applied in several previous cases and later on approved by evaluation bodies.
No particular remarks.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

Maize 65 and the control maize were grown at five locations in the US in the 2006 season.
No remarks

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

Comments/Questions of the expert(s)

*Comment 1*

If differences were found, these were minor relative to their natural variability, and they were not consistent across sites, and they were within the range of values found in literature.
This was the case for amino acids, fatty acid composition, anti-nutrients, minerals and vitamines, except one and this is copper (see table 6 and 8). However, it remains within the literature range in mg/kg DW according to the 1987 range of Watson.
In table 6 however, the value of 6.91 mg/kg DW for the MON89034x1507xNK603 was not only higher than controls (1.60 mg/kg DW) but also higher than the reference range (1.51-3.42 mg/kg DW). No obvious reason for this was given throughout the text, even if this shouldn't probably be a problem.

*Comment 2*

As in previous applications, maize 65 is compared with the control maize. The OECD document is followed with respect to the selection of constituents. In case statistical differences are found results are compared with the ILSI and other literature data.
This approach has been accepted is previous applications.

Nutrients analysed include:

- proximates,
- fibre including ADF,NDF,TDF; to my knowledge this approach is not widely used in the EU in the field of human nutrition,
- carbohydrates are assessed "by difference"; also not very relevant in human nutrition,
- minerals, vitamins, fatty acids, amino acids, anti-nutrients and secondary metabolites are well covered.

My major comment is that the OECD document needs an upgrading. This basis document was approved several years ago and is widely used in the assessment of genetically modified foods and feed.

Particularly in the field of human nutrition there are some new developments in our knowledge about relevant nutrients. This is not reflected in the OECD document. I made this observation already several times. The dietary fibre case is a typical example.

The applicant conclude that the proposed maize 65 is compositionally equivalent to conventional maize.
I do not question this conclusion but the methodology applied could be more “up to date” and more convincing.

### D.7.4 Agronomic traits

Comments/Questions of the expert(s)

*Comment 1*

No comments

*Comment 2*

No comments

### D.7.5 Product specification

Comments/Questions of the expert(s)

*Comment 1*

No comments

*Comment 2*

No comments

### D.7.6 Effect of processing

Comments/Questions of the expert(s)

*Comment 1*

No comments

*Comment 2*

Taking into account the compositionally equivalence it is not expected that there would be any difference in processing according to the dry or wet milling process.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

Well-documented. Estimates were made for humans and animals.
No further comments.

*Comment 3*

It is expected that maize 65 will replace part of the maize used as food or feed.
No further comments

### D.7.8 Toxicology

Comments/Questions of the expert(s)

*Comment 1*

- No homology with known toxins for Cry1A.105, Cry1F and Cry2Ab2 or PAT-protein (phosphino-thricin-acetyltransferase) expressed in MON89034x1507xNK603 maize.
- No indication for any toxicity in vivo in acute toxicity tests with doses many times higher than normal uptake by man in the highest possible scenario.
- NK603 maize is resistant or tolerant to glyphosate, the active component in Roundup.

The phosphonomethyl-glycine blocks the activity of 5-enolpyruvylshikimate 3-phosphate synthase or EPSPS, which is a key enzyme in the shikimic pathway leading to the formation of aromatic amino acids (tyrosine, phenylalanine, thryptophane) in plants, bacteria and fungi, but not in animals. Why then in some books or dictionaries is a low toxicity in animals mentioned ? Has the enzyme EPSPS other known functions ? Or is the term “low toxicity” misused ?

*Comment 2*

| Protein | Product | Mean (µg/g dwt) | SD | Range |
|---|---|---|---|---|
| Cry1Ab.105 | MON 89034 | 2.8 | 0.4 | 1.7 - 3.5 |
| | MON 89034 × 1507 × NK603 | 4.5 | 0.73 | 3.4 - 5.8 |
| Cry2Ab2 | MON 89034 | 5.6 | 1.1 | 2.7 - 7.1 |
| | MON 89034 × 1507 × NK603 | 5.1 | 1.3 | 1.9 - 6.3 |
| Cry1F | 1507 | 3.15 | 0.63 | 2.43 - 4.58 |
| | MON 89034 × 1507 × NK603 | 3.03 | 0.51 | 2.37 - 4.09 |
| PAT | 1507 | ND | NA | ND – ND |
| | MON 89034 × 1507 × NK603 | ND | NA | ND – ND |
| CP4 EPSPS | NK603 | 6.7 | 1.3 | 4.9 – 8.8 |
| | MON 89034 × 1507 × NK603 | 8.5 | 2.0 | 5.4 – 11 |

The protein levels in MON 89034 x 1507 x NK603 are comparable to those in the parental lines (MON 89034, 1507 and NK603).

*Comment 3*

The stacked event MON89034x1507xNK603 is produced by conventional breeding.
MON89034 maize was assessed by EFSA and EFSA adopted a positive scientific opinion on the safety (EFSA, 2008).
1507 maize was assessed by EFSA and EFSA adopted positive scientific opinions on the safety (EFSA, 2004; EFSA, 2005).
NK603 maize was assessed by EFSA and EFSA adopted positive scientific opinions on the safety (EFSA, 2003a; EFSA, 2003b).

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

A detailed description of the safety aspects of the Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins is given in the corresponding toxicology section of the respective applications for

authorization of MON 89034, 1507, and NK603 ((Monsanto Company (2006); EFSA-GMO-NL-2007-37); Pioneer Hi-Bred International, Inc. and Mycogen Seeds c/o Dow AgroSciences LLC (2007) EFSA-GMO-RX-1507; (Monsanto Company (2005) EFSA-GMO-NL-2005-22/EFSA-GMO-RX-NK603 – Section D.7.8, Pages 152, 38 and 90 - respectively).

Moreover, the Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS have already been assessed by EFSA and considered as safe for humans and animals (EFSA, 2003a; EFSA, 2003b; EFSA, 2004; EFSA, 2005; EFSA, 2008).

*Comment 2*

The stacked event MON89034x1507xNK603 is produced by conventional breeding. The newly expressed proteins were thoroughly assessed in previous dossiers.

Moreover, updated information was provided (bioinformatics analysis) for Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins, showing that there is no structural similarity to known toxins or other biologically active proteins that could cause adverse effects to humans or animals.

The potential for interaction among the Cry1A.105, Cry2Ab2, and Cry1F proteins was assessed by Levine et al. (2008) in 7-day diet-incorporation bioassays. The results showed that combining MON89034 maize and 1507 maize by conventional breeding did not alter the combined activity of MON89034 maize and 1507 maize.

No further comments.

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

Comments/Questions of the expert(s)

*Comment 1*

Not applicable

*Comment 2*

No further comments.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

**Compositional Analyses (Lundry et al., 2007)**
MON89034x1507xNK603 maize was compared to a conventional control maize with comparable genetic background (XE6001) and in addition was compared to 14 conventional reference maize hybrids (USA, one growing season, 5 field sites, glyphosate/glufosinate-ammonium use), and to literature.
No further comments: We agree that the observed differences (33) between MON89034x1507xNK603 maize and the control XE6001 can be regarded as not biologically relevant. All values fell within the range of values for the reference substances analyzed in this study and/or within the range of values for commercial maize in the ILSI crop composition database and/or the literature. Well-conducted study.

No further comments.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

a) 42-day feeding study in broiler chickens (Davis, 2008).
**The study of Davis (2008) mentions 9 different treatments and the use of 900 animals (100 per treatment). In this same study, the following articles were used:**

**Test Article:** **MON 89034 × TC1507 × NK603,**

**Control Article:** **Conventional control, XE6001,**

**Reference Articles:**
**Golden Harvest H9166**
**Dekalb DKC61-50**
**Pioneer 33N29**
**Willcross 3103**
**Willcross 3123**
**Golden Harvest H8920**

**These are only 8 treatments. What is the 9$^{th}$ one? (Possibly another test article was tested simultaneously).**
**In this same study, the following statement is made:**

**"The drum weight (expressed as % of chilled carcass weight) for male birds fed MON 89034 × TC1507 × NK603, although slightly higher than that for birds fed diets containing the control or any of the reference corn lots, appears to be a chance occurrence."**

**What's the reasoning behind this conclusion?**

b) 90-Day rat feeding study ().
Not performed. No further testing is needed.

*Comment 3* **(Vanhhooren)**

**42-day poultry feeding study (Davis, 2008)**
In this study the equivalence of the diet containing MON89034x1507xNK603 maize was compared to a diet containing conventional control maize with comparable genetic background (XE6001), and in addition was compared to 6 diets containing commercial reference corn. We agree with the applicant that there were no biologically relevant differences in broiler performance, carcass yield, or meat composition between broilers fed diets containing MON89034x1507xNK603 maize and those fed diets containing the conventional control maize. No adverse effects were observed. Well-documented and well-conducted study.

No further comments.

### D.7.9 Allergenicity

Comments/Questions of the expert(s)

*Comment 1*

No indication of any allergenicity and no characteristics of the newly expressed proteins in MON89034x1507xNK603 maize to known allergens; no sharing of immunological relevant sequence homology.

*Comment 2*

**Assessment of the allergenicity of the newly expressed proteins.**
According to the data currently available, Cry1A.105, Cry 2Ab2, Cry 1F, PAT, and CP4 EPSPS are unlikely to be allergenic.
For information, Cry1A.105, Cry 2Ab2, Cry 1F and CP4 EPSPS were shown in previous dossiers to display some sequence identity with actinidin, the major allergen of kiwi, Cop c 1, a mushroom allergen, Der p 7, a minor allergen of mite, and Der f 2, another allergen of mite, respectively. The level of identity was nevertheless inferior to the 35% threshold defined in the FAO/WHO guidelines.
It should also be emphasized that proteins of the Cry family are suspected of having adjuvant properties (Calderon et al. 2007). This has been firmly demonstrated for Cry 1Ac (Vasquez et al. 1999, Vasquez-Padron et al. 1999, Moreno Fieros et al. 2003, Esquivel-Perez et al. 2005) that shows high identity with Cry 1A.105. The consequence of the presence of such immuno-stimulants in a plant destined to human consumption is not known, particularly whether this may elicit sensitization (and

which type of sensitization) against the co-ingested maize proteins. The presence of several proteins of the Cry family in the same plant may multiply the adjuvant effect.
Therefore, it may be relevant to study the immune responses against maize antigens in mice fed this GMO maize.

On page 40 of the present technical dossier, the applicant states that “the proteins lack structural similarity to known allergens”. This sentence should be modified since it may mislead the reader. Indeed, only the primary structure of the proteins, namely the amino acid sequence, was compared with that of known allergens. Neither the secondary structure, nor, most importantly, the three-dimensional structure was studied. The sentence should read: “the proteins lack sequence similarity with known allergens”.

**Assessment of the allergenicity of the whole GM plants or crops.**
The applicant did not assess the allergenicity of the GM plant. By so doing, the applicant follows the EFSA GMO panel who consider that assessment of the allergenicity of the whole plant is not necessary if this plant is not listed in the official allergen list available in the frame of the EU regulations regarding labeling of food. Maize is not listed.
Nevertheless, the reviewer feels that, due to the introduction of the new traits as described in the application, over-expression of endogenous proteins, among them possibly the maize allergens already described, may occur. Therefore, it seems relevant to analyze whether the expression levels of known maize allergens is increased in the genetically modified maize grains or to analyze whether the overall allergenicity of the modified maize 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.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

The mortality in the trial with poultry (Davies et al., 2008) is rather high, which might have been interfering with the experimental grouping. It is not possible to calculate the power of the statistical analysis because the standard deviation is not reported per treatment group.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

### D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

*Comment 1*

No questions

### D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

*Comment 1*

No questions

### D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

### D.9.6 Effects on human health

Comments/Questions of the expert(s)

*Comment 1*

Safety for humans based on:

- Lack of acute toxicity based on mouse gavage studies.
- Rapid digestion in simulated gastric fluid
- No homology with known protein toxins

No homology with known allergens

### D.9.7 Effects on animal health

Comments/Questions of the expert(s)

*Comment 1*

No questions
Safety based on same reasons as 9.6 and further confirmed on feeding study in broiler chickens

### D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

## D.11. ENVIRONMENTAL MONITORING PLAN

### D.11.1 General

Comments/Questions of the expert(s)

*Comment 1*

No questions

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

Comments/Questions of the expert(s)

*Comment 1*

No questions, and hardly relevant in scope of the application.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions, and hardly relevant in scope of the application.

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

Comments/Questions of the expert(s)

*Comment 1*

No comments

### D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

*Comment 1*

No comments

## References


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EFSA (2003b) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (Question No EFSA-Q-2003-002). The EFSA Journal 9:1-14.

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EFSA (2005) Opinion of the Scientific Panel on Genetically Modified Organisms on an application for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds.

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## Annex I – Minority declaration of L. Flandroy (22/12/2010)

Taking into account the data available on this file, **I consider** ( as a member of the Belgian Biosafety Consultative Council ) **that no conclusions can be drawn on the safety of this GMO** which is a triple stacked event ( maize MON89034 x 1507 x NK603 ) .

Indeed, as a nutritional/toxicity assessment of the whole plant, only a 42-days study on one animal species ( chickens ) was conducted that was not taken into account by EFSA " *because of relevant deviations from good Agricultural Practices*" in the performed tests.

It is widely recognized, included by EFSA, that toxicological assessment of stacked genes cannot be based exclusively neither on corresponding single events assessments neither on conclusions on potential or improbable interactions between the proteins encoded by the stacked transgenes. ( This, because of potential unintended and unforeseeable changes induced in the GM plant through the genetic engineering technology ).

In contrast with EFSA statement in this file, I consider that the comparison of the compositional analysis of the GMO with that of its control(s) does not allow to conclude in "substantial equivalence" with the non-GMO counterpart plant.
Beside the fact that only a complete chemical analysis could conclude in "similarity" , the EFSA's reference document of OECD describing the substances to be analyzed in order to be allowed to conclude in "substantial equivalence" is dated from 2002. On the basis of present scientific and methodological evolution, several other compounds relevant for health safety (nutritionally:ex. carbohydrates and fibers; and/or toxicologically:ex.: hydroxamic acids and derivative compounds, some of them having mutagenic potentials) known to be present in maize, should be measured in a health safety assessment of GM maize, and with different methods (ex.: methods allowing to specify soluble and insoluble fibers – having different digestibility and physiological effects- , allowing to define the composition of carbohydrate fraction, now that more and more attention for the health is given to the type of carbohydrates present in human food), as repeatedly mentioned by Belgian experts assisting the BAC.
To reflect those remarks of the Belgian experts, the Belgian Biosafety Consultative Council made this observation for these compounds in a series of GMOs files since ~ 5 years (for "fibers" and more recently for hydroxamic acids) , asked EFSA to consider this point since almost 4 years ( in at least 3 written addressed documents – in June 2007, June 2008 and September 2010 - and several bilateral and European meetings ) without real taking into account of these elements by EFSA till now.
In addition, the Codex Alimentarius concerned document, to which EFSA refers, clearly states that "compositional equivalence" ( as performed following EFSA' s requirements ) is only a *starting point* for further assessment of GM plants: thus, compositional analysis can reveal compositional differences between a GM plant and its non-GM counterpart, that should be further investigated for their biosafety concern; but "compositional equivalence" is not a sufficient risk assessment in itself allowing to conclude in the safety of a GM plant.

"Agronomic characteristics" of this GMO followed on only one season ( too short to take into account e.g. reaction to different climatic stresses, and leaving moreover some uncertainties in present results ) also do not allow to conclude in "equivalence".

Allergenicity was not tested in the risk assessment. Following current knowledge, maize can induce rare but dangerous allergenic reactions, the level of which could potentially be enhanced through unexpected changes induced by the transgenic modification and by the stacking of several "transproteins" having between ~ 25 % ( Cry1A.105 of MON89034 : similarity with Kiwi allergen ) and 30 % ( CP4 EPSPS of NK603: similarity with Dermatophagoïdes sp., cause of many allergies, at least by inhalation, in our regions) homology with known allergens - , as has been repeatedly mentioned by a Belgian expert assisting the BAC, in this and various other files ( and in particular in file EFSA/GMO/NL/2007/39 for the precisions on homologies with known allergens and in file EFSA/GMO/CZ/2008/62 for anaphylactic reactions to maize allergens ). The BAC suggests to follow potential allergenicity in the monitoring phase, as suggested by another of the 4 Belgian experts assisting the BAC in this file. The monitoring plan described by the notifier however does not involve medical or veterinary professional individuals or networks.