



Secretariaat  
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O./ref.: WIV-ISP/BAC/2009\_881

**Title:** Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/UK/2005/20 from Pioneer Hi-Bred under Regulation (EC) No. 1829/2003

## Context

The application EFSA/GMO/UK/2005/20 was submitted by Pioneer Hi-Bred on 19 September 2005 for the marketing (import and processing) of the glyphosate, glufosinate and insect resistant genetically modified 59122 x NK603 maize for food and feed uses under Regulation (EC) No. 1829/2003<sup>1</sup>.

The application was officially acknowledged by EFSA on 20 June 2007. 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, 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). Ten 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 EFSA net on 21 September 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 19 November 2008 (The EFSA Journal, 2008, 874, 1-34)<sup>2</sup>, and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 2 December 2008 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.

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

<sup>1</sup> 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)

<sup>2</sup> See: <[http://www.efsa.europa.eu/EFSA/efsa\\_locale-1178620753812\\_1211902200229.htm](http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902200229.htm)>

line 59122 (EFSA/GMO/NL/2005/12) and maize line NK603 (C/ES/00/01) are taken into account in this advice. The Biosafety Advisory Council formulated a positive advice for both single events<sup>3</sup>.

## Scientific evaluation

### 1. Environmental risk assessment

According to the Biosafety Advisory Council, no major risks were identified concerning the environment<sup>4</sup>.

### 2. Molecular characterisation

With regard to the molecular characterisation, the Belgian experts demanded more detailed information on the intactness of the inserts and the flanking regions in 59122 x NK603. The additional information received was considered as sufficient.

### 3. Food/feed safety assessment

3.1. Following the comments submitted by the Belgian experts, the Biosafety Advisory Council considers that even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document<sup>5</sup>, 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.a. With regard to the comparative analysis, the Biosafety Advisory Council noticed that the hybrids used in comparative compositional/expression/agronomic studies are not the same as the ones used in animal trials: feeding trials were conducted with a higher stack, 59122 x 1507 x NK603.

3.2.b. The specific hybrid constitution of 59122 x NK603 is unclear which makes it not possible to assess the repeatability of tests. Although these confusing situations do not by definition provoke performance differences, it is considered as scientifically incorrect. Additional information of the applicant did not take away these irregularities.

3.3 The Biosafety Advisory Council observes that the allergenicity of the whole GM maize has not been evaluated. The introduction of the transforming DNA might interfere with the expression levels of other maize proteins, including allergens. Therefore, it might be relevant to analyze whether the expression levels of allergens is increased and to carry out IgE binding studies.

<sup>3</sup> Advice of BAC on maize line 59122: BAC\_2007\_SC\_536; Scientific evaluation of SBB on mandate of BAC of maize line NK603: IPH/1520/GMCROPFF/2003-0767.

<sup>4</sup> 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.

<sup>5</sup> OECD, 2001. Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients. ENV/JM/MONO(2001)15.  
<http://www.olis.oecd.org/olis/2001doc.nsf/c5ce8ffa41835d64c125685d005300b0/>

## 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 notifier to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

- a) Agrees with the GMO panel of EFSA that no major risks concerning the environment were identified;
- b) Concludes that the dossier has some major scientific shortcomings. Although it is possible that these shortcomings may not jeopardize the risk assessment substantially, the Biosafety Advisory Council considers the shortcomings as unacceptable;
- c) Can not give an advice in absence of additional data regarding hybrid constitution of 59122 x NK603 and a precise description of the material used in each test.

In addition, the Biosafety Advisory Council recommends:

- 1) To adapt the OECD consensus documents to include the analysis of dietary fibre in the compositional analysis of food;
- 2) To consider introducing assessment of allergenicity of the whole GM crops in the frame of the revision of the EFSA guidance document on Food/Feed safety assessment.



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

*Annex : Full comments of experts in charge of evaluating application EFSA/GMO/UK/2005/20 and comments submitted on the EFSAnet (ref: BAC\_2007\_PT\_575)*



**Secretariaat  
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N./réf. : WIV-ISP/BAC/2007/PT\_575  
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**Compilation of comments of experts in charge of  
evaluating the application EFSA/GMO/UK/2005/20  
and  
Comments submitted on the EFSA net on mandate of  
the Biosafety Council**

**Mandate for the Group of Experts:** mandate of the Biosafety Advisory Council (BAC) of 29 June 2007

**Coordinator:** Prof. Dirk Reheul

**Experts:** Pascal Cadot (Consultant), Eddy Decuypere (KUL), Leo Fiems (ILVO), Rony Geers (KUL), Lieve Gheysen (UGent), Jean-Pierre Maelfait (UGent), Robert Renaville (FUSAGx), Peter Smet (Consultant), Frank Van Breusegem (VIB), Johan Van Waes (ILVO)

**Domains of expertise of experts involved:** Genetics, genetic engineering, genome analysis, toxicology, animal nutrition, immunology, alimentary allergology, ecology, plant-insect relations, nature conservation, biosafety research, agronomy, herbicide tolerance, maize

**Secretariat:** Didier Breyer, Adinda De Schrijver, Martine Goossens

## INTRODUCTION

Dossier **EFSA/GMO/UK/2005/20** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **59122xNK603 maize** for food and feed applications under Regulation (EC) 1829/2003.

The application has been officially acknowledged by EFSA on 20 June 2007.

The scope of the application is:

- GM plants for food use
- Food containing or consisting of GM plants
- Food produced from GM plants or containing ingredients produced from GM plants
- GM plants for feed use
- Feed produced from GM plants
- Import and processing (Part C of Directive 2001/18/EC)
- Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

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

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

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Comments placed on the EFSA net are indicated in grey.

## List of comments received from the experts

### A. GENERAL INFORMATION

Comments/Questions of the expert(s)

#### *Comment 1*

The fact that:

- on the hand 59122 x NK603 was obtained by traditional breeding methods between progeny of two genetically modified maize lines, and that no new genetic modification has been introduced in 59122 x NK603 maize

- on the other hand:

- NK603 maize was considered as safe as conventional maize and that it therefore 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, 2003)

- the submission of an application for authorisation of genetically modified 59122 maize and derived food and feed under Regulation (EC) N° 1829/2003, and the conclusion that 59122 maize is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health or the environment (EFSA, 2007)

may be an advantage with regard to the evaluation of the application of 59122 x NK603 maize. This dossier is characterized by a holistic, integrative approach.

#### *Comment 2*

According to the dossier the scope of application does not include the authorization for the cultivation of 59122 x NK 603 maize seed products in the EU. It can however be worth to give some remarks on the different topics, dealing with cultivation and survivability of seeds, in the case that the applicant should ask in the near future for an extension for the scope of cultivation.

So as agronomical expert I will also give some comments in this questionnaire, related to cultivation and the agronomical value.

#### *Comment 3*

Under f): proposal for labelling in accordance with articles 13 and articles 25 of regulation (EC)1829/2003. In the case of GMO's, food and/or feed containing consisting of GMO's & proposal for the labelling of 59122 x NK603 maize food according to articles 12 and 13, 24 and 25, 4, B6; all contain the sentence "the 59122 x NK603 maize does not give rise to any ethical or religious concerns".

It is proposed to omit every time "ethical concern" for the following reason: it is not because inserts in 59122 x NK603 do not contain human or animal genes, or because of no differences in composition, food or feed value, absence of toxicity or allergenicity ... that there may be no ethical concerns. Ethical concerns may arise from a certain view on nature and human impact on it, based on subjective reasons originating from a certain view on nature and mankind, and not only based on objective arguments of safety. Even if these arguments giving rise to ethical concerns are completely subjective the ethical concerns are nevertheless real and have to be taken into consideration in a democracy if they arise in a substantial part of the population.

*Comment 4*

No comments

*Comment 5*

No comments or questions.

*Comment 6*

The modified maize has been presented as more resistant to glufosinate-ammonium and glyphosate herbicides. What's the level of this resistance?

Because the modified maize is presented as more resistant to glufosinate-ammonium and glyphosate, toxicity studies has to be realized to determine the residues level of these two herbicides in 59122xNK603, indeed more herbicides will be applied on 59122xNK603 than on normal maize.

As this GMO is more resistant it allows higher amounts of herbicides to be used on crops, what about the persistence in the environment and/or contamination of groundwater.

Even if the two parents of the hybrid GMO 59122xNK603 were safe this does not prove that the hybrid is safe as there could be interactions between the transgene proteins That's why toxicity analyses on the real hybrid GMO are necessary.

As 59122xNK603 will enter in the food chain as normal maize it'll probably also enter in the diet of mothers and kids. Therefore toxicity studies are lacking on gravid animals to assess possible teratogenic effects as well as the effects on neonates.

Maize is usually consumed all over the year and doesn't present a seasonal ingestion so that humans and animals will be exposed to 59122xNK603 for long periods of time even all life long. The duration of toxicity assays are therefore too limited and should be prolonged for more that 90 days to assess chronic effects.

Scientists do not consider similar things as equal so that Pioneer Hi-Bred International, Inc. It can not be assumed that 59122xNK603 is safe because similar to wild type maize.

*Comment SBB:* The toxicity of herbicides and their possible persistence in the environment are covered by Directive 91/414/EEC<sup>1</sup>

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

Comments/Questions of the expert(s)

*Comment 1*

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<sup>1</sup> Council Directive of 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

No comments

*Comment 2*

No comments

*Comment 3*

No comments or questions.

## **C. INFORMATION RELATING TO THE GENETIC MODIFICATION**

Comments/Questions of the expert(s)

*Comment 1*

What exactly is the difference between the plant EPSPS and the EPSPS from Agrobacterium CP4 so that glyphosate, the active component in Roundup, does not block CP4-EPSPS but does so with the plant EPSPS ?

*Comment 2*

No comments

*Comment 3*

No comments or 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)

*Comment 1*

As a result of the production of 59122 x NK603 maize by traditional breeding methods, the 59122 and NK603 inserts are integrated into the nuclear genome of 59122 x NK603 maize as shown by molecular characterization by Southern Blot and by sequence analysis. No questions.

*Comment 2*

No comments

*Comment 3*

No comments or questions.

*Comment 4*

Pioneer Hi-Bred International, Inc. conclude that “there were no statistically significant differences between 59122xNK603 and non-GM control maize with comparable genetic background that fell outside the normal ranges of variation for commercial maize”. In the annex 5, statistical differences can be observed in some amino acids, minerals, vitamins, ... These differences in maize composition can not be justified by a “in the range of historical values” this is not a scientific method, values should always be confronted with the control of the same trial.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

Appropriate molecular approaches should be used to assess intactness of the stacked transgene events. Southern blot analysis has been performed but for the NK603 insert, the enzyme/probe combination only can detect internal fragments. Therefore, this analysis does not confirm the intactness of the borders of the insert in the stacked hybrid maize. According to the text (D.2.e), PCR on the borders has been done and the data were submitted to JRC but no further information is given in this dossier. Upon checking the JRC website, I have found the data on real-time PCR, confirming that the 3' end of the insert is most likely unchanged (PCR method works with similar efficiency, but there is no data on fragment length or sequence). However, no data is provided on the other side of the insert.

*Comment 3*

The information on the inserted sequence + flanking ) regions in NK603 was not assessed as the reader is referred to an earlier notification. It would be very informative to provide a short summary of this information (as done for 59122) in both the technical dossier and the summary file.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

The guidelines for stacked events demands an expression analysis during the life cycle and a comparison of transgene protein levels in the line with stacked events compared to the parent lines for the relevant tissues. The applicants give a comparison of the protein levels in grains from herbicide treated stacked and parent lines. As this corn will not be grown in Europe and will only be imported, the only relevant tissue also for feed is indeed the grain, for which the data are provided in this dossier.

*Comment 3*

No comments or questions.

**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*

Remarks concerning the survivability of seeds of maize. In the dossier it is mentioned that seed cannot survive outside managed agricultural conditions. Furthermore it is mentioned that freezing temperatures have an adverse effect on germination. The minimum temperature for germination of 8 to 10°C restricts maize survival and reproduction capabilities mainly to the Southern European geographical zones. This is correct but from our experience maize seeds can survive in the soil during a not so severe winter. It can happen that out of full ears, fallen on the ground at harvest and after labouring of the land, covered with soil, some seeds survive and give plantlets during the next season. So here in the case of GMO-plants it will be necessary to have a follow up of the fields in the next year to detect for surviving plants. This information is only relevant if at a certain moment the scope would be extended to cultivation in Northern and Western Europe with moderate to cold winter conditions.

*Comment 2*

No questions

*Comment 3*

No comments

*Comment 4*

No comments or questions.

## 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*

The stability analysis in annex 3 shows the CP4EPSPS gene (from the NK603) to be present in all (85) the analysed plants from the 59122xNK603 stacked hybrid. The transgenes from 59122 and their corresponding proteins are missing in many of the samples and the applicants discuss this as 'confirming the plants were segregating for the 59122-insertion'.

*Additional comment from coordinator: The diagrams representing how the hybrids are made (Annex 12) are speculative. Besides, according to these diagrams the transgenes end in different genetic backgrounds. As a consequence the "hybrids" that are used in animal trials are not the same as the "hybrids" that are used in agronomic performance+composition+expression trials. Although these confusing situations do not by definition provoke performance differences, it is a scientifically incorrect procedure.*

*It is impossible to make a commercial hybrid if one works as indicated in the diagrams; as a consequence we expect the commercial hybrid to be different again from the tested material.*

*So the commercialized product will not be genetically equal to the tested products. Again this is scientifically not correct.*

If these are hybrid seeds, shouldn't all samples have both parents' contribution? If these plants are progeny from the selfed hybrid with the stacked events shouldn't both transgene inserts be segregating? I would like the applicants to be more clear on this (I may have overlooked an explanation for this in the huge dossier, but it would be logical that this is explained in the results and discussion of the analysis itself).

The guidelines for stacked events recommend to analyse the molecular stability over several generations, but this is not necessary if multiplication is not the aim.

### *Comment 3*

No comments or questions.

### *Comment 4*

SNPs, sequencing and Microarray method exist to evaluate modification of gene expression. These new technologies which are much more accurate must be introduced in the panel of tests used to determine the eventual effects of a GMO in tissue.

## **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*

It is explained that there are few to no possibilities for transfer of genetic material to other plants in case of unintended release of 59122 x NK603 maize e.g. via spillage during transportation of grain since the scope of this application does not include authorization for the cultivation of 59122 x NK603 maize seed products in EU.

There are few possibilities of survival of maize, no vegetative propagation, no possibilities for crossing with other plants in Europe, no weedy characteristics. In addition, since there are no differences with non-GM maize, spreading is easy to control with conventional agricultural methods. Together with low survivability of seeds, all information points to the absence of any possibility for escaping of the gene constructs to other plants.

### *Comment 2*

No comments

### *Comment 3*

No comments or questions.

## **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 safety of the Cry34Ab1, Cry35Ab1, and PAT proteins in 59122 maize (EFSA 2007), and CP4 EPSPS protein in NK603 (EFSA, 2003) has previously been assessed, for which positive opinions were issued.

### *Comment 2*

In this chapter it is mentioned that 59122 x NK603 maize was compared to non-GM maize with comparable background. Wherever possible publicly available data on commercial maize has also been used in the comparisons. What does it mean? The 59122 x NK603 is tolerant to glyphosate and glufosinate-ammonium. So I think it is not possible to compare with commercial varieties, unless they are also tolerant to glyphosate and glufosinate-ammonium (= are also genetically modified).

### *Comment 3*

Observed significant differences across location, especially for some amino acids, between 59122 x NK603 maize and non-GM maize, were not consistently observed anymore on a per location based analysis.

Moreover, this non-consistency was differential in the 3 cases, namely when GM maize was treated with glyphosate and compared to non-GM control maize, or when GM maize was treated with glufosinate, respectively glyphosate + glufosinate and compared to non-GM control maize. Therefor it is concluded correctly that there is substantially equivalence between 59122 x NK603 maize and non-GM commercial maize, regardless of herbicide treatment.

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

Comments/Questions of the expert(s)

### *Comment 1*

No questions

### *Comment 2*

Data from literature are not good base for comparison, the only valid control is the non-GMO maize collected in the same period, same region. These comparisons shows significant differences (annexe 6) between GMO and controls which can not be justified by a literature mean value.

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

Comments/Questions of the expert(s)

### *Comment 1*

It is not clear if Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS proteins were extracted from 59122 x NK603 maize or obtained from bacterial production, as it often occurs for these proteins. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004).

### *Comment 2*

No comments

#### **D.7.4 Agronomic traits**

Comments/Questions of the expert(s)

*Comment 1* ( with additions from the coordinator)

The 59122 x NK603 maize was tested in the USA and Canada during the 2003 growing season; another genetically different version was tested in Chile during 2002-2003. The results obtained confirmed that there are no unexpected agronomic differences between the 59122 x NK603 maize and non-GM-maize with comparable background. Results of 1 testing season are never conclusive since there is no opportunity to test potential year effects. And the material tested in Nord America was genetically not the same as the material tested in South America.

So my remark: The results are only based on 1 year trials and the year effect can be given significant effects. And furthermore: what does it mean: unexpected agronomic differences?

*Comment 2*

No comments

#### **D.7.5 Product specification**

Comments/Questions of the expert(s)

*Comment 1*

No comments

#### **D.7.6 Effect of processing**

Comments/Questions of the expert(s)

*Comment 1*

No comments

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

Comments/Questions of the expert(s)

*Comment 1*

No comments

## D.7.8 Toxicology

Comments/Questions of the expert(s)

### *Comment 1*

- no homology with known toxins for Cry34 x Cry35 x PAT-protein expressed in 59122-NK603 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 (“worst”) 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 and thryptophane) in plants, bacteria and fungi, but not in animals. Why then in some text books or dictionaries a low toxicity in animals is mentioned ? Has the enzyme EPSPS other known functions ? Or is the term “low toxicity” misused ?

### *Comment 2*

No new genetic modification has been introduced in 59122xNK603 maize. This maize has been obtained from traditional breeding methods between progeny of genetically modified 59122 and NK603 maize.

- ◆ 59122 maize expresses the Cry34Ab1 and the Cry35Ab1 proteins, which act against corn rootworm larvae. Besides these traits, it also expresses the PAT protein which confers tolerance against glufosinate-ammonium herbicide. All three proteins have previously been tested for acute toxicity. Results seemed to be negative.
- ◆ NK603 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene which encodes for the CP4 EPSPS protein which confers tolerance against glyphosate herbicide to the plant. Previous testing of this protein in mice, revealed no acute toxicity.

### *Comment 3 (with details added by the coordinator)*

The transgene proteins PAT, CP4EPSPS and Cry34Ab1 + Cry35Ab1 were tested separately and not together; this does not give the opportunity to have data of possible interactions between these proteins.

Only acute studies were done, some effects can only be seen after a long period of exposure so chronic studies are needed. Moreover these studies were done with the two parents of the hybrids and not with the hybrid.

-under application,

The data were not collected by independent labs!

The GMO is considered as safe as both its components are but NK603 was not considered as safe by an independent committee of experts (Crie-Gen)

Comment SBB: The Crie-Gen study as been published in:

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE, (2005) Differential effects of glyphosate and roundup on human placental cells and aromatase . Environmental Health Perspectives 113 (6).

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

Comments/Questions of the expert(s)

*Comment 1*

At what temperature are PAT and CP4 EPSPS proteins inactivated? The Cartagena Protocol (Part III of the dossier, p. 122) mentions an activation of Cry34Ab1 and Cry35Ab1 proteins at 90°C.

*Comment 2*

Safety assessment of the newly expressed proteins was based on:

- protein specificity
- no homology with known protein toxins
- very quickly digested in vitro and therefore very little chance the intestine would be exposed to possible feed allergens, if any present
- no acute toxicity

*Comment 3*

Further testing for acute toxicity of these proteins, all of which being present in 59122xNK603 maize, is **not required**.

*Comment 4*

It is well-known that the pesticides are endocrinal disruptors. In clinical investigations, endocrine measures are considered routine measures in assessing patient health. In this dossier there are no mentions of any endocrine tests! Endocrine axis is the first to be disrupted in illness so that they can not be removed from a toxicity study.

Cry3Bb1 has toxic effects on insect intestine. There are no scientific demonstration that this protein has no effects on human and animal intestine.

The safety assessment is based on comparison with existing toxins but if Cry3Bb1 is not similar to any toxin known this does not mean that it is not toxic!

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

Comments/Questions of the expert(s)

*Comment 1*

Not applicable

*Comment 2*

As more herbicides will be spread on cultures it is likely that more residues would be present on crops, what about glyphosate and glufosinate-ammonium residues?

What's the impact of these high glyphosate and glufosinate-ammonium quantities on hormonal status of animals and humans?

Comment SBB: The toxicity of herbicides and their possible persistence in the environment are covered by Directive 91/414/EEC<sup>2</sup>

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

### **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 was performed by using **59122x1507xNK603 maize**.

First, it was checked whether protein content in **59122xNK603 maize** is similar to that in the 59122 and NK603 maize respectively. This was indeed the case for the Cry34Ab1, Cry35Ab1 and PAT proteins.

	Cry34Ab1 (ng/mg dry w.)	Cry35Ab1 (ng/mg dry w.)	PAT (ng/mg dry w.)	CP4 EPSPS (µg/g fresh w.) (ng/mg dry w.)
59122	19.5 – 84.8	0.48 – 4.8	0.000 – 0.94	-
NK603	-	-	-	6.9 – 15.6 (fresh) ??? (dry)
59122x NK603	21.8 – 53.6	0.530 – 3.31	0.000 – 0.440	2.601 – 13.94 (fresh) 3.06 – 16.4 (dry)

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<sup>2</sup> Council Directive of 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

For the CP4 EPSPS protein it was rather difficult to find precise data. The data indicated in red are based on fresh weights and are mentioned in the technical dossier (UK/2005/20; part I; pg.17). **Where do these data come from?** In annex 5 of dossier 59122xNK603 only the values based on dry weight are provided (UK/2005/20; annex 5; tables 91-92-93).

In this study no adverse effects were detected due to the use of **59122x1507xNK603 maize** as animal feed.

**A 13-week feeding study in the rat** is not included. Such a study **should be performed** since synergistic effects cannot be excluded beforehand.

*Comment summarized by the coordinator:*

Contents of CP4 EPSPS proteins presented in technical dossier (UK/2005/20; part I, pg. 17) are expressed as concentrations on fresh weight, while in annex 5 (tables 91-92-93) data are expressed on dry weight. Where do the fresh weights come from ?

*Comment 3*

The feeding study was not realised with the GMO considered in this application, but the applicant claims that it is relevant for the safety assessment.

This acute study is too short to observe long term effects. A chronic study should be conducted. Further study should be conducted to understand the effect of the GMO on abdominal fat and kidneys.

Rem SBB : The feeding study was conducted with 59122x1507xNK603 maize. The applicant says it is relevant for the safety assessment of 59122xNK603 maize.

### **D.7.9 Allergenicity**

Comments/Questions of the expert(s)

*Comment 1*

FAO/WHO (2001) proposes pepsin degradation as a method for the evaluation of allergenicity of genetically modified foods.

Furthermore, the similarity of amino acids with known allergens was studied as described by FAO/WHO (2001), where a cross-reactivity between the expressed protein and a known allergen has to be considered when there is:

- 1) more than 35 % identity in the amino acid sequence of the expressed protein, using a window of 80 amino acids and a suitable gap penalty, or
- 2) identity of 6 contiguous amino acids. However, there is no proof that a six or eight amino acid match is predictive in the bioinformatics section. A number of people now recommend not performing the 6-8 amino acid match.

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used to test the digestion of Cry34Ab1 and Cry35Ab1 proteins (Annex 8i), PAT protein (Annex 9) and CP4 EPSPS protein (Part I of the dossier, p. 37). It has been shown that a rapid in vivo degradation of Cry proteins (Cry1Ab) does not always occur (Chowdhury et al., 2003). The fact that major allergens with high percent allergenicity were not necessarily more resistant to SGF or SIF digestion than allergens with low percent allergenicity renders the use of SGF and SIF digestibility difficult as a tool to distinguish

potential food allergens from nonallergenic proteins (Fu et al., 2002). Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.

#### *Comment 2*

See remarks under 7.8;

No indication of any allergenicity and no characteristics of the newly expressed proteins in 59122 x NK603 maize to known allergens; no sharing of immunological relevant sequence homology.

#### *Comment 3*

Pioneer argues that the donor organisms have no history of causing allergy but as these organisms are soil bacteria it's obvious that these organism were not included in a normal human diet so that couldn't have provoked allergies.

Moreover Pioneer claim no allergenicity for the new proteins because they don't share aminoacids sequences with known allergens but again these proteins are new in human alimentation and so there is a need of specific scientific studies. So that no allergenicity has been registered for these proteins as that haven't been part of human diet before.

#### *Comment 4*

As mentioned by the applicant, Cry34Ab1, Cry35Ab1, and PAT are not likely to be allergenic proteins. CP4 EPSPS has already been demonstrated to share some sequence similarity with Der f 2, a major allergen of the mite *Dermatophagoides farinae*. A recent report, however, concluded that there is no evidence of increased allergenic potential for CP4 EPSPS (Hof et al., 2007).

As rightly mentioned by the applicant, food allergy to maize is rare. Some allergens have been determined (Pastorello et al., 2003; Pasini et al., 2002), and new allergens might be described in the near future (Weichel et al., 2006). Although the newly introduced proteins are not likely to be allergens, and although both parent plants do not seem to have increased allergenicity, their breeding gives rise to what can be considered as a new plant, with potentially new molecular interactions. Theoretically, this might cause some modification in the expression levels of some maize proteins, including allergens. Therefore, it is the feeling of the reviewer that it might be relevant to analyze whether the allergenicity of the whole new plant is increased, compared to its traditional counterpart.

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

Comments/Questions of the expert(s)

#### *Comment 1*

For each of the locations the phytase concentration was nominally higher for GM maize than for the control maize, while the difference was significant for 2 locations. The mean of the 6 locations was also significantly higher (Table 33; Annex 5). Phytic acid is considered as an anti-nutritive constituent,

which is important for monogastric animals. As a result, extra phosphorus should be incorporated into the diet.

It is a pity that the poultry feeding study was not conducted with 59122 x NK603 maize, but as performance, carcass yield and meat composition, and health in broiler chickens in comparison with conventional maize hybrids were not detrimentally affected by 59122 x 1507 x NK603, containing Cry1F beside Cry34Ab1, Cry35Ab1, PAT, and CP4 EPSPS protein, (Annex 11), it can be assumed that 59122 x NK603 maize is safe as animal feed due to its nutritional equivalence. However, the relatively high phosphorus content may have masked the effect of high content of phytic acid. Moreover, it is nowadays a common practice to add phytase for environmental reasons, so that the total phosphorus content in diets for monogastric animals can be reduced.

I did not find any information dealing with the in vitro organic matter digestibility of 59122 x NK603 maize. This is a rapid technique that can provide interesting information. Based on the chemical composition and the in vitro organic matter digestibility, the metabolic and net energy can be estimated, yielding extra information for pigs and ruminants. In the poultry feeding study feed efficiency was not different, which may be an indication of a similar digestibility of GM and control maize.

*Comment 2*

No comment

*Comment 3*

Significant differences in chemical composition were found even if in a range of historical concentrations this is not accepted as scientific demonstration to compare data from different studies.

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

Comments/Questions of the expert(s)

*Comment 1*

No comment

*Comment 2*

As no long term toxicity studies has been done, we can not exclude long term effect of OGM consumption. That's why it is required a follow-up of the GM food post-market.

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

Comments/Questions of the expert(s)

*Comment 1*

The mechanism of interaction between GM plant and target organism, hence the action mechanism of Cry34Abs and CryAb1 is very important to understand since it is linked with its specificity. Cry proteins are activated by proteases and disrupt the insect gut wall via pore formation mediated by binding to specific gut receptors.

Cry35Ab1 alone has no effect while Cry34Ab1 has an effect but this is strengthened by Cry35 in a mixture; however the exact best mixture is not mentioned or known.

What about these gut receptors ? Anything known about their nature ? Their prevalence in other species (or other larvae, since in adult corn rootworm they seem to be absent as well).

Although the mechanism of action based on specific gut receptor for Cry proteins is very crucial, also for the specificity of action, information given on this topic is rather limited.

As the application for the authorization of 59122 x NK603 maize is for feed & food use, not for seed & plant propagation or cultivation in EU, this aspect is therefore perhaps not so important as if it were for cultivation authorization.

*Comment 2*

Indeed, not applicable.

## **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*

Not relevant in scope of this application

*Comment 2*

Provided information: sufficient.

### **D.9.2 Selective advantage or disadvantage**

Comments/Questions of the expert(s)

*Comment 1*

It is mentioned that maize is highly domesticated and cannot become established as a feral species outside the agricultural environment. How must we interpret the term “agricultural environment”: southern Europe conditions (warm and dry) are more favourable for maize plants compared to northern Europe (cold and wet in spring).

*Comment 2*

Not relevant in scope of this application.

*Comment 3*

Provided information: sufficient.

**D.9.3 Potential for gene transfer**

Comments/Questions of the expert(s)

*Comment 1*

The possibility of gene transfer seems to be very low to negligible because it is not intended to use 59122 x NK603 maize for cultivation.

*Comment 2*

Not relevant in scope of this application

*Comment 3*

Provided information: sufficient.

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

Comments/Questions of the expert(s)

*Comment 1*

- see remarks under D8 as for the Cry proteins
- no questions as for CP4-EPSPS

*Comment 2*

Indeed, not applicable.

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

Comments/Questions of the expert(s)

*Comment 1*

- same remarks as under D9.4
- see also remark under D9.5 for Maize 59122

Comment SBB: see comment 2 under D9.5 in document BAC/2007/PT/522 (Comments of experts in charge of evaluating the application EFSA/GMO/NL/2005/23) :

" What is really understood by non-target organisms ?  
Are other arthropoda, or even other coleoptera also "non-target organisms"? Or is this restricted to vertebrates ?

If it is stated that there is a limited persistence in soil, it means that D T50 is less than 3.2 days, but how limited is that for soil organisms ?

What about ratios of Cry 34/Cry 35 for other insects or invertebrates ? Whenever in the technical dossier the interaction of Cry 34 Ab1 and Cry 35 Ab1, proteins and non-target insects is mentioned, a total mixture is given but without any specification of the ratio Cry 34/Cry 35 except for honey bees where it is specified.

It is excluded that in other ratios the activity may be different for different insect species. Again, knowledge about the specificity or occurrence of the gut receptors for Cry-proteins in insects would be helpful."

*Comment 2*

Provided information: sufficient.

#### **D.9.6 Effects on human health**

Comments/Questions of the expert(s)

*Comment 1*

No questions; no effects on human health of 59122 x NK603 maize products nor of its specific proteins (Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS) are likely.

#### **D.9.7 Effects on animal health**

Comments/Questions of the expert(s)

*Comment 1*

Based on toxicological studies and the poultry feeding study (Annex 11) a safe use of 59122 x NK603 can be assumed.

*Comment 2*

No questions; no effects are likely (see also report on 59122 x NK603) maize.

*Comment 3*

Six trials with mice and rats are reported. In the following reports no control group was included: Annex 8c, d, e, k. The feeding study with rats (Annex 8f) reported different results for males and females. According to the available data, the sample size for male rats should have been 100 instead of 12 in order to detect a significant difference (Berndtson, 1991), while for the females sample size is sufficient and the difference in growth rate should be significant based on the calculated confidence intervals. Annex 15 reports a trial including control groups, but only means without standard error are available, which does not allow to estimate the power of the statistical analysis.

No remarks on the broiler trial reported in Annex 11.

*Comment moved by the coordinator under D.7.10*

## **D.9.8 Effects on biogeochemical processes**

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

Provided information: sufficient.

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

Comments/Questions of the expert(s)

*Comment 1*

In this paragraph it is mentioned again that the scope of application does not include cultivation of maize plants of 59122 x NK 603 maize in the EU. Nevertheless I give here some remarks in the case that the applicant should ask in the near future for an extension for the scope of cultivation. In the framework of the EU- regulation 2002/53 a new variety have to be submitted to DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use) tests before the variety can be commercialised. The new variety has to be compared with the best existing standard varieties. So my question here is : can the GM- maize be incorporated in normal VCU trials, for example treated with specific herbicides for maize and will the agronomical value be the same as tested in trials, where herbicides for which the variety is tolerant were used?

*Comment 2*

No questions

*Comment 3*

Indeed, not applicable.

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

Comments/Questions of the expert(s)

*Comment 1*

No questions

*Comment 2*

Provided information: sufficient.

## **D.11. ENVIRONMENTAL MONITORING PLAN**

### **D.11.1 General**

Comments/Questions of the expert(s)

#### *Comment 1*

If seeds were imported by train containers for making food and feed, some monitoring has to be done to control if there are no maize plants along the railway roads. As already mentioned under a moderate winter seeds of maize can survive and can give plantlets in the next spring; so these plants have to be destroyed.

#### *Comment 2*

No questions

#### *Comment 3*

We support the view of ACRE in its annual report of 2006 (ACRE, 2007; p.42) that provision of the detailed arrangements for general surveillance post-market monitoring plans should be made a condition of any consent.

Although resistance to insect attack is not the only factor preventing maize to grow outside the agricultural environment, the (indeed low) possibility of the establishment of maize protected against insect larvae in the wild in Europe should be a point of particular interest in a more detailed general surveillance plan.

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

Comments/Questions of the expert(s)

#### *Comment 1*

Hardly relevant here in scope of the application

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

Comments/Questions of the expert(s)

#### *Comment 1*

Hardly relevant here 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 comment

#### **D.11.5 Reporting the results of monitoring**

Comments/Questions of the expert(s)

*Comment 1*

No comment

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