Bioveiligheidsraad
Conseil de Biosécurité

15-06-2009

Secretariaat
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O./ref.: WIV-ISP/BAC/2009_966

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/UK/2005/21 from Pioneer Hi-Bred under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2005/21 was submitted by Pioneer Hi-Bred on 19 September 2005 for the marketing (import and processing) of the glyphosate and glufosinate tolerant and insect resistant genetically modified 59122 x 1507 x NK603 maize for food and feed uses under Regulation (EC) No. 1829/2003[1].

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 EFSAnet on 21 September 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 3 April 2009 (The EFSA Journal, 2009, 1050, 1-32)[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 9 April 2009 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 line 59122 (EFSA/GMO/NL/2005/12), maize line 1507 (C/ES/01/01) and maize line NK603

[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/EFSA/efsa_locale-1178620753812_1211902438985.htm>

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

(C/ES/00/01) are taken into account in this advice. The Biosafety Advisory Council formulated a positive advice for each single event[3]. All three events are authorised for food and feed uses[4].

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[5].

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 1507 x NK603. The additional information received was considered as sufficient.

3. Food/feed safety assessment

3.1. Assessment of compositional analysis.
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[6], 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 and recommends the adaptation of the OECD consensus documents accordingly.

3.2 Assesment of allergenicity
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 maize proteins, producing potential allergens. Therefore, it might be relevant to analyze whether potential allergens do occur.

[3] 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; Scientific evaluation of SBB on mandate of BAC of maize line 1507: IPH/1520/GMCROPFF/2006-0839.

[4] See Community Register <http://ec.europa.eu/food/dyna/gm_register/index_en.cfm>

[5] 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.

[6] OECD, 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea Mays*): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites. ENV/JM/MONO(2002)25. http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)5

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

a) no major risks concerning the environment were identified;

b) No major risks for human and animal health were identified.

In addition, the Biosafety Advisory Council recommends:

1) To include the analysis of dietary fibre in the compositional analysis of food and to adapt the OECD consensus documents accordingly;

2) To evaluate the allergenicity of the whole GM crop.

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

Annex: Full comments of experts in charge of evaluating application EFSA/GMO/2005/21 *and comments submitted on the EFSAnet (ref: BAC_2007_PT_576)*

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21/09/2007

Secretariaat
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N./réf. : WIV-ISP/BAC/2007/PT_576
Email. : bac@sbb.ihe.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2005/21 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 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/2005/21** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **59122x1507xNK603 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 EFSAnet 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 1507 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 1507 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)
  - EFSA (2005) considers that 1507 maize will not have an adverse effect on human and animal health or the environment in the context of its proposed use.

may be an advantage with regard to the evaluation of the application of 59122 x 1507 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 1507 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

No questions; for the labelling, I refer to comments on EFSA dossier UK/2005/20 on Maize 59122 x NK603. (see comment 3 under point A in document ref. BAC_2007_PT_575)

Comment 4

No comments

Comment 5

No comments or questions.

Comment 6

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.

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 59122x1507xNK603, indeed more herbicides will be applied on 59122x1507xNK603 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.

In this dossier, 59122x1507xNK603 was often declared to be safe as it was obtained from normal breeding of three GMOs but some controversies has emerged about the safety of one of these (NK603).

Even if the three GMOs from which 59122x1507xNK603 has been obtained were safe this does not signify that this one is safe as there could be interactions between the proteins inserted. That's why the toxicity analyses has to be done on this GMO.

As 59122x1507xNK603 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 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 59122x1507xNK603 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 59122x1507xNK603 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[1]

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

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.

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

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

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, …

Pioneer Hi-Bred International, Inc. conclude that "there were no statistically significant differences between 59122x1507xNK603 and non-GM control maize with comparable genetic background that fell outside the normal ranges of variation for commercial maize" instead in the annexe 6, statistical differences can be observed in 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.

Concerning the Southern blot analyses, the plasmid controls do not always behave as expected: the CP4EPSPS is much weaker than would be expected from a positive copy control, the cry34 control is even invisible in figure 11 (annex 2); in contrast the pat and cry1F controls are often much stronger. Do the applicants have an explanation for this?

Comment 3

PartI / P15: I did not find back the details of the results that back up the statements made in the last paragraph on p15, concerning the detailed analysis of the DNA flanking regions at both the 5' and 3' borders of the 1507 insert. Therefore I could not fully assess the information on the sequences actually inserted (including flanking genomic regions) for the 1507 insert. However, the reader is referred to Annex 5 (the sequence itself), to the Annex 1b folder in which a summary of the characteristics of the 1507 maize is described by an EFSA panel, and to previous authorizations to place the 1507 maize to the market. Therefore 1507 event is considered safe.

The information on the inserted sequence + flanking maize genomic regions in NK603 was not assessed, because for this information, reference is made to an earlier notification.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Cry34Ab is present at 26.1 to 68.7 ng/mg dry weight and Cry35Ab1 at 0.84 to 3.49ng/mg dry weight, which is comparable to the expression in 59122 maize.
Cry1F is present at 0.81 to 3.56 ng/mg dry weight, which is comparable to the expression in 1507 maize (1.2 to 3.1 ng/mg dry weight).
CP4 EPSPS in 59122 x 1507 x NK603 maize is comparable in the expression in NK603 maize.
Molecular equivalence and identical copy number in 59122 x 1507 x NK603 maize and those present in 59122, 1507 and NK603 maize respectively could be expected as no new genetic modification has been introduced in 59122 x 1507 x NK603 maize which was obtained from traditional breeding methods between progeny of the single genetic modified maize strains. It also indicates that no fusion proteins are formed.

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

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 differences in agricultural characteristics as for reproduction, dissemination, survivability or other parameters. No field data differences.

Comment 3

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

Why is one of the three transgene inserts (1507) segregating and the others not in the seeds of the hybrid with stacked events? Are the non-segregating inserts homozygous in this line? Furthermore it is not really explained how this triple stacked 'hybrid' was obtained ('through breeding'). I would like the applicants to be a bit more clear on this point.

The diagrams in dossiers EFSA/GMO/UK/2005/20 and 21 are exactly the same, while the hybrids differ.

The diagrams representing how the hybrids are made (Annex 13) 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.

Comment 3

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.

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), Cry1F (EFSA2005) 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 1507 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 1507 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

Both commercial maize as well a comparative assessment with non-GM control maize with comparable background genetics (1W2, 61B and 05F) as the 59122 x 1507 x NK603 maize has been used as the baseline.

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

Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS proteins were bacterially produced (Annex 16). Practically it may be impossible to obtain a sufficient amount of plant derived protein, but It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004).

Comment 2

Statistically significant differences between 59122 x 1507 x NK603 maize and the non-GM control maize were observed for phosphorus, potassium, oleic- and linoleic acid, tryptophan, methionine and Vit E.

However, on a per location basis, these differences were not consistently observed.
All values in 59122 x 1507 x NK603 maize grain and non-GM control maize were within reported literature ranges.
Therefore, it is correctly concluded as for equivalency of GM and non-GM control maize and of grain from commercial maize.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

The 59122 x 1507 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 1507 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 effets. And the material tested in Nord America was genetically not the same as the material tested in South America.

was tested in the USA and Canada during the 2003 growing season. The results obtained confirmed that there are no unexpected agronomic differences between the 59122 x 1507 x NK603 maize and non-GM-maize with comparable background.
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 comment

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comment

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions; the anticipated intake of the expressed proteins is several orders of magnitude below levels shown to have no effect in laboratory toxicology testing.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

- no homology with known toxins for Cry1F, Cry34 x Cry35 x PAT-protein expressed in 59122 x 1507 x 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, phenylalamine 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 59122x1507xNK603 maize. This maize has been obtained from traditional breeding methods between progeny of genetically modified 59122, 1507 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.

- 1507 maize expresses the Cry1F protein - which protects against certain lepidopteran pests - as well as the PAT protein. Previous testing of the Cry1F protein revealed no acute toxicity.

Comment 3

The effect on the growth rate and feed intake of rats was tested. The number of animals used in the trial was sufficient for the female rats, but not for the male rats, due to a different variability within both sexes (Berndtson, W.E., J. Anim. Sci. 69, 67-76, 1991).

Comment 4

The transgene proteins PAT, CP4EPSPS, Cry1F 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 proteins inserted were tested separately and not together which doesn't 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 three GMO used to made 59122x1507xNK603 but not with the GMO under application, these acute and chronic studies are needed.

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 (Crii-Gen).

The broiler study demonstrated that they are toxic effects of GMOs as there is a significant difference between GMOs and control for kidney yield and abdominal fat.

Comment SBB: The Crii-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

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 2

Further testing for acute toxicity of these proteins, all of which being present in 59122x1507xNK603 maize, is **not required**.

Comment 3

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?

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 **59122x1507xNK603 maize** is similar to that in the 59122, 1507 and NK603 maize respectively. This was indeed the case for the Cry34Ab1, Cry35Ab1, PAT and Cry1F proteins.

	Cry34Ab1 (ng/mg dry w.)	Cry35Ab1 (ng/mg dry w.)	PAT (ng/mg dry w.)	Cry1F (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	-	-
1507	-	-	< LLOQ	1.2 – 3.1	-
NK603	-	-	-	-	6.9 – 15.6 (fresh) ??? (dry)
59122x1507xNK603	26.1 – 68.7	0.840 – 3.49	0.000 – 0.180	0.81 – 3.56	3.61 – 13.43 (fresh) 4.25 – 15.8 (dry)

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/2005/21; part I; pg.20). **Where do these data come from?** In annex 6 of dossier 59122x1507xNK603 only the values based on dry weight are provided (UK/2005/21; annex 6; table 54).

In this study no adverse effects were detected due tot 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/2005/21; part I, pg. 20) are expressed as concentrations on fresh weight, while in annex 6 (table 54) data are expressed on dry weight. Where do the fresh weights come from ?

Comment 3

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.

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 know 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 non allergenic 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 1507 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 amino acids 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 allergenecity 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. Cry1f, due to very low similarity with Der p 7, a mite allergen, has been further investigated

by the applicant, but does not seem to have allergenic potential (Ladics et al., 2006). 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 (Hoff 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 the parent plants do not seem to have increased allergenicity, their breading 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

I did not find any information dealing with the in vitro organic matter digestibility of 59122 x 1507 x NK603 maize. This is a rapid technique that can provide interesting information. Based on the chemical composition and the 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.
Annex 12 (p.22) mentioned the presence of CP4 EPSPS protein in 2 out of 6 control diet samples. So, the control diet were not really a negative control. On the other hand, the fact that these results did not show a detrimental effect on the chickens may provide some guarantee. This may be a reflection of a practical situation where novel proteins in the diet may not only come from 59122 x 1507 x NK603 maize, but also from GM soybean meal, wheat, ...

Comment 1

Significant differences in chemical composition where 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

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.
For Cry1F the action mechanism is similar to the interactions between B-thuringiensis Cry-proteins and target organisms.
δ-endotoxins produced as protoxins dissolve in alkaline conditions of insect gut and are processed by proteases to release the active toxin, the aminoterminal part equal to the Cry1F protein. These bind to receptors of apical villi of insect midgut cells, with oligomerization of toxin and pore formation resulting in lysis and cell death and finally insect dead.
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 1507 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 where 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 1507 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)

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 1507 x NK603 maize products nor of its specific proteins (Cry1F, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS) are likely.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

Based on the toxicological studies (Annex 1b and 1c, Annex 9a to 9k) and the poultry feeding study (Annex 12) a safe use of 59122 x 1507 x NK603 can be assumed.

Comment 2

No differences in all parameters for 59122 x 1507 x NK603 maize compared with non-GM maize with comparable genetic background was found except for kidney weight in female broilers and for abdominal weight in male broilers where 59122 x 1507 x NK603 maize-fed broilers had lower values for both parameters than non-GM-fed broilers. However values were within the tolerance range calculated (lower and upper limits of a 95% tolerance interval on 99% of the population of broilers fed

the different diets) for kidney and abdominal fat weight from chickens fed the 3 commercial maize diets (33P66, 33J56, 33R77).
Therefore, occasional differences, once in females then in males, but with no consistency and within normal organ weight tolerance zone as described above, can be neglected and considered as not due to the GM-maize.

Comment 3

No remarks on the poultry feeding study.

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 1507 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 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 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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