Biosafety Advisory Council

01-02-2006

Secretariat

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Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/UK/2004/06** of Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2004/06 was submitted by Monsanto in November 2004 for the marketing (import and processing) of the insect-protected glyphosate-tolerant genetically modified hybrid maize MON863 x NK603 for food and feed applications under Regulation (EC) No. 1829/2003. It has been officially acknowledged by EFSA on 14 January 2005.

On the same date EFSA started the 3 months formal consultation of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms (GMOs) being part of the products).

In the frame 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. Six experts answered positively to this request. The comments received from the Belgian experts (see Annex I for an overview of all the comments) were synthesised by the coordinator and put on the EFSAnet on 14 April 2005 (see Annex II for comments actually placed on the EFSAnet).

The opinion of EFSA's scientific panel on GMOs was adopted on 6 July 2005 (The EFSA Journal, 2005, 255, 1-21)[1].

[1] see: http://www.efsa.eu.int/science/gmo/gm_ff_applications/more_info/703_en.html

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On 21 December 2005 the opinion of EFSA was forwarded to the Belgian experts and they were given access to the additional data received from the applicant on request of EFSA. The experts were invited to give comments and to react in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

Scientific evaluation

The critical points raised by the Belgian experts can be summarised as follows (for the scientific evaluation we refer to annex I):

1. Molecular characterisation

It was noted during the consultation period that concerning the DNA flanking the insert in MON863 there remain unanswered questions about what really happened during insertion. There is a possibility that mitochondrial DNA co-integrated with the MON863 gene construct during the transformation process. In this case, at the junctions between nuclear chromosomal DNA and the mitochondrial DNA, new genes could have been formed, with the potential for the production of new proteins with unintended long-term effects. It was therefore recommended to continue the sequencing of flanking regions until plant nuclear genomic sequences were found.
In its opinion on MON863 and MON863 x MON810 (The EFSA Journal, 2004, 49, 1-25), the EFSA GMO Panel states that: "The molecular analysis does not differentiate between the integration of insert DNA within a region of mitochondrial DNA that is already present in the nuclear genome and the acquisition of this organelle DNA as part of the primary integration during transformation. The integration of organellar DNA within plant nuclear genome is established as a normal phenomenon and the Panel considers that the resolution of this distinction would not significantly impact on the safety assessment done."
The Biosafety Advisory Council did not receive reactions on this point from the Belgian experts.

2. Food/feed safety assessment

2.1 Toxicological assessment of the whole GM food/feed

Two experts noted during the consultation period that there was a need for additional data to confirm the safety assessment of the hybrid MON863 x NK603. In particular, an additional 90-day rat feeding study, including complete endpoints (biochemical, haematological, histological), with the hybrid MON863 x NK603 to exclude any adverse effect on human health was asked for.
This concern was relayed by EFSA to the applicant. A 90-days toxicity study with rats fed with MON863 x NK603 maize, was provided and assessed by the GMO Panel of EFSA. It was concluded that a 90-day sub-chronic rodent study with MON863 x NK603 maize indicated that there are no adverse effects from its consumption.

The Biosafety Advisory Council did not receive reactions on this point from the Belgian experts.

2.2. Nutritional assessment of GM food/feed

During the consultation period it was stated that the feeding trials should have included more animals per treatment to increase the power of the statistical analysis or sensitivity of the trials.
This comment has not been retained by the EFSA GMO panel nor forwarded to the applicant as a request.
The opinion of the Belgian experts that it is difficult to detect an effect on mortality when the background noise (i.e. the overall mortality) is too high is nevertheless maintained.

4. Environmental risk assessment and monitoring plan

No major issues were raised during the consultation period.

Conclusion

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

1) EFSA did take into account some (but not all) remarks of the Belgian experts. As a result, the notifier was asked to deliver complementary information (e.g. additional information on an extra 90-day sub-chronic feeding trial with rats – topic D.7.8.4). The delivered information underpinned and consolidated the EFSA advice that there were no adverse effects of the consumption of the genetically modified maize hybrid MON863 x NK603.
2) The Belgian Biosafety Advisory Biosafety council continues to disagree with EFSA on topic D.7.10: Nutritional assessement of GM food/feed. The feeding trials presented by the notifier, included a small number of animals (all trials). Trials reported in file 18175 – rats- and in file 18163 –broilers- show high threshold differences between the different treatments. Trials with broilers, reported in files 17243 and 18163 show an overall high animal mortality; mean values and variation were not reported in file 17243. As a result of these weaknesses, the power of the statistical analysis or the sensitivity of the trials is too low to draw scientifically sound conclusions. In addition it is difficult to detect an effect on mortality when the background noise, i.e. the overall mortality, is too high.

In absence of correct data concerning the nutritional assessment of this GM maize, the Belgian Biosafety Advisory Council can not support the positive advice of EFSA for the application EFSA/GMO/UK/2004/06.

p.o.

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

Annex I : Comments of experts in charge of evaluating application EFSA/GMO/BE/2004/06 (ref: BAC_2005_PT_233)

Annex II: Belgian comments submitted on the EFSAnet on mandate of the Biosafety Council (print-out of EFSAnet pages)

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Bioveiligheidsraad
Conseil de Biosécurité

April, 14th, 2005

Secretariaat
Secrétariat

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Comments of experts in charge of evaluating the application EFSA/GMO/UK/2004/06
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council of February 2th, 2005
Coordinator: Dirk Reheul (UGent)
Experts: Philippe Baret (UCL), Jacques Dommes (ULg), Rony Geers (KUL), Jean-Pierre Maelfait (Instituut voor Natuurbehoud), Hadewijch Vanhooren (KUL), Michel Van Koninckxloo (Haute Ecole Provinciale du Hainaut Occidental)
Domains of expertise of experts involved: genetics, population genetics, horizontal gene transfer, GMO traceability, biosafety research, ecology, plant-insect relations, nature conservation, sustainable development, agronomy, animal feed, toxicology and immunology
Secretariat: Martine Goossens, Adinda De Schrijver

INTRODUCTION

Dossier **EFSA/GMO/UK/2004/06** concerns a notification of Monsanto for the marketing (import and processing) of the genetically modified hybrid maize MON863xNK603 for food and feed applications under Regulation (EC) No. 1829/2003.
The notification has been officially acknowledged by EFSA on 14 January 2005. The deadline for posting comments on the EFSAnet by the Member States is 14 April 2005.

This document gives an overview of all the comments received by the experts involved in the safety evaluation of application EFSA/GMO/UK/2004/06. The experts were asked to structure their comments according to 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; see attachment). Comments placed on the EFSAnet are indicated in grey.

LIST OF COMMENTS

A. GENERAL INFORMATION

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

Comment 1

The non-modified parental organism is a current maize hybrid variety.
This kind of maize are currently cultivated, processed and used for food and feed in E.U.
There is no risk for the human health and the environment related to the release in the environment of the non-modified maize.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAIT(S) AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comment 1

This application concerns a genetically modified maize referred to as MON863 x NK603. It was produced by crossing two genetically modified maize lines, referred to as MON863 and NK603 (parental lines).

MON863 produces an insecticide protein (Cry3Bb1) from the bacterium *Bacillus thuringiensis*. This plant is therefore protected against certain coleopteran insect pests. The gene construct introduced in this plant contains a gene coding for the Cry3Bb1 protein and a marker gene coding for neomycin phosphotransferase II (conferring tolerance to some aminoglycoside antibiotics). The gene coding for Cry3Bb1 is made of :

- a modified 35S promoter (from cauliflower mosaic virus) allowing high level of transcription in roots
- the sequence of the 5' untranslated leader of the chlorophyll a/b binding protein mRNA from wheat (improving translation level of the mRNA)
- an intron of the rice actin 1 gene (improving transcription level)
- the coding sequence of a variant of the Cry3Bb1 protein of *B. thuringiensis* subsp. *kumamotoensis*
- the terminator of the wheat heat shock protein 17.3

The selection marker gene is made of :

- the 35S promoter
- the coding sequence of neomycin phosphotransferase II (*NptII*) from the Tn5 transposon of *Escherichia coli*
- a portion of the coding sequence of the ble protein from the Tn5 transposon (non functional, part of the operon containing *NptII*)

- the terminator of the nopaline synthase gene from *Agrobacterium tumefasciens*.

NK603 produces the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) conferring tolerance to the non-selective broad spectrum herbicide Roundup. The gene construct introduced in this plant contains two genes coding for two variants of the EPSPS protein. The first EPSPS gene is made of :

- the rice actin 1 promoter
- an intron of the rice actin 1 gene (improving transcription level)
- the sequence of a chloroplast transit peptide from *Arabidopsis thaliana* (directing the CP4 EPSPS protein to the chloroplast)
- the coding sequence of the EPSPS enzyme from *Agrobacterium sp*. strain CP4
- the terminator of the nopaline synthase gene from *Agrobacterium tumefasciens*.

The second EPSPS gene is made of :

- a modified 35S promoter (from cauliflower mosaic virus) with a double enhancer region
- an intron from the maize hsp70 gene (improving transcription level)
- the sequence of a chloroplast transit peptide (*ctp2*) from *A. thaliana* (directing the CP4 EPSPS protein to the chloroplast)
- the coding sequence of a variant of the EPSPS enzyme from *Agrobacterium sp*. strain CP4 (Pro instead of Leu at position 214)
- the terminator of the nopaline synthase gene from *A. tumefasciens*.

The MON863 x NK603 hybrid was obtained by crossing inbred lines that are homozygous in the MON863 or the NK603 insert. Therefore the MON863 x NK603 hybrid should contain one copy of the MON863 insert and one copy of the NK603 insert.

Comment 2

MON 863 x NK603 comprises traditionally bred maize varieties, produced by the combination of two genetically modified maize lines. MON 863 x NK603 thereby inherits an effectively combines the two introduced traits of agronomic interest, which where individually contained in the parental lines.

MON 863:
On 2 April 2004, EFSA issued a favourable opinion on the safety of MON 863 (EFSA,2004). The approval of MON 863 maize under Regulation (EC) N° 258/97 and Directive 2001/18/EC is pending.

NK603
On 25 November 2003, FSA issued a favourable opinion on the safety of NK603 (EFSA, 2003) which resulted in the approval of this maize for import, processing and feed use in the EU on 19 July 2004. The approval of NK603 under Regulation (EC) N° 258/97 is pending.

The application (dossier and appendix) gives a complete description of the result of the genetic modification (methods used for the genetic modification, copy number of inserts in MON 863 x NK603, information on the expression and the stability of the insert …)

Composition :
Statistical analyses showed that 99.7% (309) of the 310 comparisons made between MON 863 × NK603 and the traditional control were either not significantly different or they were within the calculated 99% tolerance interval for a population of commercial reference hybrids. The

remaining single statistical difference was only noted at one site. In addition, this one value was within the range of values reported in previous Monsanto maize compositional studies, and, therefore, this single statistical difference was not considered to be biologically meaningful.
Based on these data, it is concluded that MON 863 × NK603 is compositionally equivalent to traditional maize.

Agronomic traits:
Observations from field trials (Carringer et al., 2004), breeding trials and from commercial cultivation show that, except for the introduced traits, MON 863 × NK603 hybrids are agronomically, phenotypically and morphologically equivalent to parental single-trait hybrids and to traditional maize.

Processing:
Using both wet and dry milling processes, maize is converted into a diverse range of food and feed products and derivatives used as food and feed ingredients or additives. MON 863 × NK603 was shown to be substantially equivalent to its parental maize MON 863 and NK603 as well as to commercially available non transgenic varieties, except for the introduced sequences and the expressed Cry3Bb1, NPTII and CP4 EPSPS proteins, which were shown to be safe for human and animal health. Therefore, when MON 863 × NK603 is used on a commercial scale as a source of food or feed, then these products are not expected to be different from the equivalent foods and feeds originating from traditional maize.

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

Comment 1

In both parental lines, the transgenic inserts are located in the nuclear genome. The copy number is claimed to be 1 per haploid genome.

To check the presence of the parental transgenic inserts and their copy numbers in the MON863 x NK603 hybrid, Southern analysis was used. DNA was extracted from the hybrid, from both parental lines, from a control non transgenic line and from a control non transgenic line spiked with plasmid PV-ZMIR13 (containing the MON863 construct) or with plasmid PV-ZMGT32 (containing the NK603 construct). It was cut by *Eco*RV. Southern blots were hybridised with a ^{32}P labeled MON863 *cry3Bb1* probe. The data presented by the applicant (fig. 4 of technical dossier) indicate that the hybrid contains the MON863 insert. Only one hybridisation signal is detected, suggesting the presence of a single copy of this insert. However the presence of additional small fragments of the insert cannot be ruled out. In parallel experiments, Southern blots were hybridised with a ^{32}P-labeled *ctp2-cp4 epsps* probe. The data presented by the applicant (fig. 5 of technical dossier) indicate that the hybrid contains the NK603 insert. Two hybridising DNA fragments (3.8 and 2.8 kb) can be seen on the blots. As *Eco*RV cuts in the NK603 insert, yielding a 3.8 and a 2.8 kb DNA fragment, this suggests the presence of a single copy of this insert. Again, the presence of additional small fragments of the insert cannot be ruled out.

As the MON863 x NK603 maize will not be used for cultivation in Europe, there is no need to assess the stability of the transgenic inserts.

Concerning the DNA flanking the insert in MON863, there are still unanswered questions about what really happened during insertion. According to the applicant, DNA sequences of about 1000 bp on the 5' end, and approximately 650 bp on the 3' end of DNA were determined and these sequences proved to be 100% identical to maize mitochondrial DNA. This observation can be interpreted in two ways. In the first one the MON863 gene construct would have been inserted in a piece of mitochondrial DNA, itself already inserted in one of the nuclear chromosomes. If this is the truth, then the molecular data gathered by the applicant are sufficient to prove that no new functional gene was formed during transformation and that there is no potential for the production of a new protein. However there is still the possibility that this piece of mitochondrial DNA co-integrated with the MON863 gene construct during the transformation process. At the junctions between nuclear chromosomal DNA and the mitochondrial DNA, new genes could have been formed, with the potential for the production of new proteins.

It is not possible to exclude any unintended long term effect that could arise from the expression of new chimaeric genes at junctions between nuclear and mitochondrial DNA (in case of co-integration). So I would recommend to continue the sequencing of flanking regions until plant nuclear genomic sequences can be found.

Comment 2 (Detection and quantification)

In the technical dossier, the applicant refers to data provided for the two parental lines MON863 and NK603. The applicant provided the full sequence of both the MON863 and the NK603 inserts (confidential information). Sequences of flanking genomic DNA were also provided. Quantitative detection procedures (real-time PCR) were also provided. With the current methods of detection, it is not possible to distinguish the line containing the stacked events from the parental lines in seed lots. This can only be done at single seed level.

For analysis of junction regions of event MON863, two primer pairs were proposed. For the amplification of the 5' junction, one primer is derived from the 5' flanking genomic sequence and the second primer corresponds to a sequence located near the 5' end of the insert in the 35S promoter. This primer pair allows the amplification of a 508 bp DNA fragment. For the amplification of the 3' junction region, one primer is derived from the 3' flanking genomic sequence and the second one corresponds to a sequence located in the cry3Bb1 coding sequence near the 3' of the transgenic insert. This primer pairs allows the amplification of a 584 bp DNA fragment. These primer pairs were used in PCR reactions on DNA samples from MON863 and from a non transgenic maize line. Fragments of the expected size were obtained from MON863. Moreover sequencing of the PCR products yielded the expected sequences. Negative controls did not yield any amplification products. These results show that these two flanking regions can be used for the specific detection and identification of the MON863 event. For quantitative detection of MON863 in MON863 x NK603 maize, the applicant also provided a real-time PCR procedure in which an 84-bp fragment of the region that spans the 5' insert-to-plant junction is amplified using two specific primers. A validated procedure was provided in JRC format.

For analysis of junction regions of event NK603, PCR analyses were carried out as for event MON863. However I did not find the precise sequence of the primers in the information provided. For the amplification of the 5' junction, one primer is derived from the 5' flanking genomic sequence and the second primer corresponds to a sequence located near the 5' end of the insert in the actin 1 promoter – intron sequence. This primer pair allows the amplification of a 982 bp DNA fragment. For

the amplification of the 3' junction region, one primer is derived from the 3' flanking genomic sequence and the second one corresponds to a sequence of the transgenic insert, apparently located in or near the *HSP70* intron. This primer pairs allows the amplification of a 3134 bp DNA fragment. These primer pairs were used in PCR reactions on DNA samples from NK603 and from a non transgenic maize line. Fragments of the expected size were obtained from NK603. Moreover sequencing of the PCR products yielded sequences with only a few differences in comparison to vector sequences (these few mismatches were shown to be due to sequencing errors of PV-ZMGT vector). These results show that theses two flanking regions can be used for the specific detection and identification of the NK603 event. For quantitative detection of NK603 in MON863 x NK603 maize, the applicant also provided a real-time PCR procedure in which an 108-bp fragment of the region that spans the 3' insert-to-plant junction is amplified using two specific primers. A validated procedure was provided in JRC format.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comment 1

The applicant provided data on the expression of the Cry3Bb1, CP4 EPSPS and NPTII proteins in maize tissues collected from MON863 x NK603 grown in field trials (different locations). Protein concentrations were assayed in forage and grains using specific ELISAs. These protein contents were compared to those measured in the parental lines. Levels of NPTII were below the limit of detection of the assay (0.21 µg/g fresh weight). Levels of Cry3Bb1 and CP4 EPSPS were not significantly different from the one measured in the MON863 and the NK603 parental lines respectively.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comment 1

MON863 x NK603 grain is unchanged compared to traditional maize in terms of invasiveness of natural environments and persistence in the environment. Importantly, there is no information to indicate that there is a potential for MON863 x NK603 to establish, persist and disperse to a greater extent than traditional maize. In cases where incidental release occurs and a MON863 x NK603 plant would establish, these plants will be easily controlled by currently available selective herbicides (except glyphosate) and by mechanical means.

Comment 2

No reasons to expect that MON 863 X NK 603 maize would spread more likely in the wild than traditional maize.

Not to be expected that the GMHP is a less poor competitor than the parental plant in natural conditions in our region.

D.5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

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

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

D.7.2 Production of material for comparative assessment

D.7.3 Selection of material and compounds for analysis

D.7.4 Agronomic traits

D.7.5 Product specification

D.7.6 Effect of processing

D.7.7 Anticipated intake/extent of use

D.7.8 Toxicology

Comment 1

MON 863 x NK603 is produced by crossing the parental single-trait maize lines MON 863 and NK603 by means of traditional breeding methods. Both of the introduced traits from the parental lines are inherited in MON 863 x NK603. This results in the combined expression of the Cry3Bb1, NPTII and CP4 EPSPS proteins in the same plant.

Comment 2

According to question 1, I am not a specialist of toxicology but I wonder whether toxicological tests are not compulsory. From page 91 (below), I understand that for some varieties toxicological tests were performed on chicken AND rats and for the present authorization, the tests were realized on chicken only. From my point of view, the rationale for this downgrading of the procedure is unclear. Feed from this plant will be used in pigs and cattle and I am unsure of the relevance of an extrapolation from chicken to pigs and cattle.

Page 91

The safety of maize containing these introduced proteins was further confirmed by repeat-dose feeding studies in the rat and in broiler chickens using MON 863 and NK603 grain containing diets (*see* Section D.7.8.4), demonstrating the absence of any toxic or pleiotropic effects linked to the genetic modifications.

Finally, the wholesomeness of the MON 863 × NK603 product, when fed to livestock animals, has been confirmed in a 42-day broiler chicken study (*see* Section D.7.10.2). Because of the high growth rate of the broiler chicken, its ability to consume high amounts of maize in its diet and the statistical sensitivity of broiler studies due to the large number of experimental units and low variability, broiler chickens are considered a highly appropriate animal model for confirming the safety of genetically modified maize lines for livestock. The results of feeding MON 863 × NK603 grain to broiler chickens show that there were no biologically relevant differences in a range of parameters tested between birds fed the MON 863 × NK603 test product, the non-transgenic control or one of five commercially available reference varieties. As a result, it was concluded that MON 863 × NK603 is as safe and as wholesome as traditional maize, as could be expected on the basis of the safety data and the compositional analyses for this maize and its parental single-trait lines.

D.7.8.1 Safety assessment of newly expressed proteins

Comment 1

Screening for structure-activity relationship, *in vitro* digestibility assays, and acute toxicity testing

The introduced proteins, the Cry3Bb1, NPTII and CP4 EPSPS proteins, were demonstrated to be safe for animal and human health. These proteins were well characterised in accordance with the applications for authorisation of MON 863 and NK603. Substantial equivalence has been established for MON 863 and NK603.
A battery of tests designed to evaluate the Cry3Bb1, NPTII and CP4 EPSPS proteins for characteristics associated with food allergens and toxins raised no concern. The Cry3Bb1, NPTII and CP4 EPSPS proteins shared no sequence homology with known toxins (other than B.t. proteins for Cry3Bb1). There is a rapid digestion of all three proteins in *in vitro* simulated gastric fluids and lack of acute toxicity for the Cry3Bb1, NPTII and CP4 EPSPS proteins, as determined by a mouse acute oral toxicity study. The proteins used for the *in vitro* digestibility testing and the acute oral toxicity testing in mice have been produced by *E. coli* and are considered to be equivalent to the MON 863 and NK603 proteins.

Comment 2

It is highly unlikely that the proteins Cry3Bb1, NPTII and CP4 EPSPS would cause adverse health effects in humans or animals.

D.7.8.2 Testing of new constituents other than proteins

Comment 1

No constituents other than the Cry3Bb1, NPTII, and CP4 EPSPS proteins, are novel. MON 863 x NK603 was shown to be compositionally equivalent to traditional maize. Agreed.

D.7.8.3 Information on natural food and feed constituents

Comment 1

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

D.7.8.4 Testing of the whole GM food/feed

Comment 1

The applicant concluded that the safety assessment for the individual proteins is not changed when combined in MON 863 x NK603, since the proteins: 1° are unlikely to interact, 2° have very different and well-documented modes of action, 3° are localized to different subcellular compartments, 4° are produced in very low quantities in MON 863 x NK603, and 5° were shown to be safe in their individual safety assessments. Furthermore, a confirmatory animal feeding experiment was conduced using MON 863 x NK603 fed to broiler chickens.

Poultry broilers feeding study

The 42-day broiler chicken feeding study using whole grain MON 863 x NK603 (Taylor et al., 2004: Report No MSL-18762) was conducted to compare the nutritional value of MON 863 x NK603 and non-transgenic control as well as additional commercial maize hybrids. The results show that there were no biologically relevant differences in the parameters tested between broilers fed the MON 863 x NK603 diet and the non-transgenic control diet.

This conclusion was consistent with the evaluation of the composition of MON 863 x NK603, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize. Relevant reports: Ridley et al., 2004: Report No MSL-19157; Ledesma et al., 2004: Report No MSL-18946.

Rat feeding study

Maize lines MON 863 and NK603 were separately tested:

In a sub-chronic (90-days) toxicity study in rats fed MON 863 maize, no consistent differences in the measured clinical, biological and histological parameters were noted for rats fed on non-GM or MON 863 maize except for some differences observed in haematological parameters, including total white blood cell, lymphocyte and basophil counts. White blood cell counts were slightly increased for the male 33% MON 863 group compared with control and reference groups. At study termination, statistically significant decreases for reticulocyte counts were observed in the female 33% MON 863 group compared with control and reference groups. Also a statistically significant decrease in individual kidney weights (males, 33% MON 863 group), and a statistically significant lower

incidence of mineralized kidney tubules was noted in female 33% MON 863 fed animals. It was accepted by EFSA that the differences found were considered not to be of biological significance.
In a sub-chronic (90-days) toxicity study in rats fed NK603 maize, no consistent differences in the measured clinical, biological and histological parameters were noted for rats fed on non-GM or NK603 maize except for slightly elevated levels of average corpuscular volume and average corpuscular haemoglobin in female rats administered with a high dose. It was accepted by EFSA that these findings were concluded as of no biological significance.

According to the applicant, it was considered that it is scientifically valid to use data from the single GM lines MON 863 and NK603 to support the safety assessment of the hybrid MON 863 x NK603. Outdoor experiments, a compositional study, and a broiler chicken feeding study performed with MON 863 x NK603 maize, support the conclusion that adverse effects are highly unlikely to occur following oral exposure to MON 863 x NK603 maize.
Although broiler chickens are the livestock animal of choice for confirming nutritional equivalence, confirmatory data for the safety assessment of the hybrid MON 863 x NK603 is needed, in particular, the need for an additional 90-day rat feeding study, including complete endpoints (biochemical, haematological, histological), with the hybrid MON863 x NK603 to exclude any adverse effect on human health (see p14 of http://www.biosafety.be/NF/GuidanceNotes/Documents/Chapter3_Toxicology.pdf). In this rodent feeding study, experimental treatments should include the GM crop and a non-GM counterpart with comparable genetic background, and a range of commercial non-GM controls. Plants should be grown under conditions that represent normal practice for the crop plant.

Comment 2

Feeding trials should have included more animals per treatment to increase the power of the statistical analysis or sensitivity of the trial in all three cases.

File 18175: trial with rats
The threshold difference between two treatments seems to be 7 to 10%, based on the observed coefficient of variation and the number of animals per treatment. Hence, more animals per treatment would have been more appropriate to increase the sensitivity of the test.

File 17243: trial with broilers
Reported mortality ranges from 3 to 7 % through treatments, which is rather high as being compared with standard practice on farms, i.e. <1%.
Mean values with variation are not reported, so that it is difficult to calculate the power of the statistical analysis.

File 18163: trial with broilers
Again reported mortality is rather high within some treatments, up to 7%.
Mean values and variation are reported, showing that differences from 10 to 15% on can be detected, which is much higher than in a farm environment. Hence, also in this case the sensitivity of the trial is too low.

D.7.9 Allergenicity

D.7.10 Nutritional assessment of GM food/feed

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

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

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

Comment 1

As the scope of the application doesn't cover seed and plant propagating material for cultivation in Europe, the risk for the environment is clearly reduced and indirect (unintended release).

MON 863:
Data collected on the environmental fate and non-target organism toxicity studies for the Cry3Bb1 proteins support the conclusion that corn event MON 863 containing Cry3Bb1.11098 protein poses no significant risk to the environment. The predicted minimal risks to the environment are consistent with described insecticidal potency data in known susceptible species (Colorado potato beetle, southern corn rootworm and western corn rootworm). MON 863 was considered to be as safe as traditional corn with respect to food, feed and environmental safety.

D.9.1. Persistence and invasiveness

Comment 1

MON 863 x NK603 will not be cultivated in EU, in case of unintended release we remind that:
There are no wild relatives of maize in Europe and the risk of genetic transfer to other species is negligible;
Maize cannot survive without human assistance and is not capable of surviving as a weed due to past selection in its evolution. Volunteer maize is not found growing in fencerows, ditches or roadsides as a weed. Although maize seed from the previous crop year can over-winter in mild winter conditions and germinate the following year, it cannot persist as a weed (Hallauer, 1995). The appearance of "volunteer" maize in fields following a maize crop from the previous year is rare under European conditions. Maize volunteers are killed by frost or, in the unlikely event of their occurrence, are easily controlled by current agronomic practices including cultivation and the use of selective herbicides.
Maize grain survival is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development (Rossman, 1949). Freezing temperatures have an adverse effect on maize seed germination and have been identified as being a major risk in seed maize production (Wych, 1988). Temperatures above 45° C have also been reported as injurious to maize seed viability (Craig, 1977).

It is concluded that MON 863 × NK603 does not differ from traditional maize with regard to reproduction, dissemination, survivability or other agronomic and phenotypic traits.

D.9.2 Selective advantage or disadvantage

Comment 1

MON 863 x NK603 will not be cultivated in EU, in case of unintended release MON 863 x NK603 has no selective advantages. Indeed the corn rootworm (Diabrotica), probably imported from

America, is now considered as a threat to maize culture only in some regions of EU. The tolerance to Roundup herbicide is not a selective advantage as maize is not found growing in EU as a weed. Furthermore there are no biologically significant differences in the reproductive capability, dissemination or survivability of MON 863 or NK603 when compared to traditional maize.

Comment 2

GMHP will not be deliberately planted. If spilled seeds germinate nearby cultivated maize fields and produce pollen that arrives on the crop, a gene transfer might occur.
Because of the low competitive abilities of maize plants in our regions in general in F2 plants in particular a spread in the wild environment is not to be expected.

Comment 3

Page 81

> Finally, in the case of germination of any spilt grain (which will be of the F2 generation) or in the unlikely case of misuse of the grain for planting, the fitness of the resulting plants would be expected to be *less* than typical, commercially
>
> - Technical dossier 81

Page 82

> available maize hybrid cultivars. Reduced fitness would result from the fact that, as with all F1 hybrid cultivar maize seed, F1 hybrid cultivars of MON 863 × NK603 do not "breed true". Consequently, the growth, development and yield of the resulting (F2) plants is variable and predominantly reduced in vigour, their morphology often resembling the less vigorous inbred lines from which the F1 seed was produced.
>
> ***d) Estimation of the risk***
>
> In conclusion, the risk of the coleopteran pest protection and the glyphosate-tolerance traits in MON 863 × NK603 to be the cause of any competitive advantage or disadvantage impacting the receiving environment is negligible.

Affirmations about reduced fitness are based on theoretical considerations without any references to the scientific literature. It seems important to me to quantify this fitness both by modeling and on the basis of on experiments.

Page 83

> As the likelihood of accidentally spilt MON 863 × NK603 kernels to germinate, establish, mature and flower is very low and as the majority of maize pollen is largely confined to short distances from the source plant, the transfer of the introduced traits to neighbouring maize plants through cross-pollination is negligible. Furthermore, in the highly unlikely case where a trait would be transferred, the risk of the insect-protection and glyphosate-tolerance traits to be the cause of any meaningful competitive advantage or disadvantage that could impact the receiving environment is negligible (*see* Section D.9.2).

This paragraph is proposed without any references to the literature. I don't understand how a risk can be described as "negligible" in absence of quantitative information. What is the scope of "negligible" in terms of probability (below 0.1 %, 1 %; 10 %)? I agree that most of the effects may be small but a major issue is the range of variation of these "negligible" effects. Most of the environmental problems occurring during the last decades are due to high impact consequences of very improbable events. I suggest asking the notifier to provide quantitative data on the risk of transfer to neighbouring maize plants and on the competitive advantage of disadvantage of the plants occurring from this transfer on the basis on the literature. In absence of convincing answer to this point, I consider that the risk should be qualified on "uncertain" and not of "negligible".

D.9.3 Potential for gene transfer

Comment 1

see D.9.1

D.9.4 Interactions between the GM plant and target organism

Comment 1

Irrelevant as MON 863 x NK603 will not be cultivated in EU.

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

Comment 1

Irrelevant as MON 863 x NK603 will not be cultivated in EU.

Comment 2

Not to be expected because a release in the field is not intended.

D.9.6 Effects on human health

D.9.7 Effects on animal health

Comment 1

MON 863 × NK603 inherits the inserts present in both the MON 863 and NK603 parental lines. The introduced protection against certain coleopteran insect pests and the introduced tolerance to glyphosate herbicide are traits of agronomic interest that are not intended to change the nutritional aspects of maize.

A thorough evaluation of the safety of the individual Cry3Bb1, NPTII and CP4 EPSPS proteins, as well as safety testing of MON 863 × NK603 expressing these proteins, indicate that it is highly unlikely that these proteins would cause adverse health effects in humans or animals.

Please see : 07_App02_CBI_Vol_I_of_II_rat_863_MSL_18175.pdf
07_App02_CBI_Vol_II_of_II_rat_863_MSL_18175.pdf

NB: Looking in those appendix to find information susceptible to confirm the allegation published by Crii-Gen in "Le Soir" 23 March 05 about an eventual blood anomaly, I found
Pp 25 of 1139 "***Hematology and Coagulation***

> There were no alterations in the hematology and coagulation data, differential leukocyte counts, or cellular morphology results that would indicate an effect from the feeding of any of the test diets. The values were generally unremarkable and comparable between the groups at Weeks 5 (coagulation not performed) and 14. The importance of four isolated statistical findings related to high-dose MON 863 could not be evaluated based solely upon the statistical criteria used. The statistical findings included the mean values in the male group for hemoglobin at Week 5 and white cell count, absolute lymphocyte count, and absolute basophil count at Week 14. While statistically significant, these mild alterations are of no biologic importance and not attributed to event MON 863".

D.9.8 Effects on biogeochemical processes

Comment 1

Irrelevant as MON 863 x NK603 will not be cultivated in EU.

Comment 2

Not to be expected because a release in the field is not intended.

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

Comment 1

Irrelevant as MON 863 x NK603 will not be cultivated in EU.

Comment 2

Not to be expected because a release in the field is not intended.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

D.11.2 Interplay between environmental risk assessment and monitoring

Comment 1

Care should be taken that no feral populations develop from spillages during transit from import zones to mills.

D.11.3 Case-specific GM plant monitoring

D.11.3 General surveillance of the impact of the GM plant

D.11.5 Reporting the results of monitoring

Application GMO-UK-2004-06 - Cpmments from Belgium (extracted from the EFSAnet)

Dirk Reheul	Belgian Biosafety Advisory Council	No	Belgium	D, 02 Information on the sequences actually inserted or deleted	The data presented by the applicant (fig. 4 of technical dossier) indicate that the hybrid contains the MON863 insert. Only one hybridisation signal is detected, suggesting the presence of a single copy of this insert. However the presence of additional small fragments of the insert cannot be ruled out. The data presented by the applicant (fig. 5 of technical dossier) indicate that the hybrid contains the NK603 insert. Two hybridising DNA fragments (3.8 and 2.8 kb) can be seen on the blots. As EcoRV cuts in the NK603 insert, yielding a 3.8 and a 2.8 kb DNA fragment, this suggests the presence of a single copy of this insert. Again, the presence of additional small fragments of the insert cannot be ruled out. As the MON863 x NK603 maize will not be used for cultivation in Europe, there is no need to assess the stability of the transgenic inserts. Concerning the DNA flanking the insert in MON863, there are still unanswered questions about what really happened during insertion. According to the applicant, DNA sequences of about 1000 bp on the 5' end, and approximately 650 bp on the 3' end of DNA were determined and these sequences proved to be 100% identical to maize mitochondrial DNA. This observation can be interpreted in two ways. In the first one the MON863 gene construct would have been inserted in a piece of mitochondrial DNA, itself already inserted in one of the nuclear chromosomes. If this is the truth, then the molecular data gathered by the applicant are sufficient to prove that no new functional gene was formed during transformation and that there is no potential for the production of a new protein. However there is still the possibility that this piece of mitochondrial DNA co-integrated with the MON863 gene construct during the transformation process. At the junctions between nuclear chromosomal DNA and the mitochondrial DNA, new genes could have been formed, with the potential for the production of new proteins. It is not possible to exclude any unintended long term effect that could arise from the expression of new chimaeric genes at junctions between nuclear and mitochondrial DNA (in case of co-integration). So I would recommend to continue the sequencing of flanking regions until plant nuclear genomic sequences can be found.
Dirk Reheul	Belgian Biosafety Advisory Council	No	Belgium	D, 04 Information on how the GM plant differs from the recipient plant in: …	No reasons to expect that MON 863 X NK 603 maize would spread more likely in the wild than traditional maize. Not to be expected that the GMHP is a less poor competitor than the parental plant in natural conditions in our region.
Dirk Reheul	Belgian Biosafety Advisory Council	No	Belgium	D, 07.08 Toxicology	Testing of the whole GM food/feed Comment 1: Although broiler chickens are the livestock animal of choice for confirming nutritional equivalence, confirmatory data for the safety assessment of the hybrid MON 863 x NK603 is needed, in particular, the need for an additional 90-day rat feeding study, including complete endpoints (biochemical, haematological, histological), with the hybrid MON863 x NK603 to exclude any adverse effect on human health. In this rodent feeding study, experimental treatments should include the GM crop and a non-GM counterpart with comparable genetic background, and a range of commercial non-GM controls. Plants should be grown under conditions that represent normal practice for the crop plant. Comment 2: Feeding trials should have included more animals per treatment to increase the power of the statistical analysis or sensitivity of the trial in all three cases. File 18175: trial with rats The threshold difference between two treatments seems to be 7 to 10%, based on the observed coefficient of variation and the number of animals per treatment. Hence, more animals per treatment would have been more appropriate to increase the sensitivity of the test. File 17243: trial with broilers Reported mortality ranges from 3 to 7 % through treatments, which is rather high as being compared with standard practice on farms, i.e. <1%. Mean values with variation are not reported, so that it is difficult to calculate the power of the statistical analysis. File 18163: trial with broilers Again reported mortality is rather high within some treatments, up to 7%. Mean values and variation are reported, showing that differences from 10 to 15% on can be detected, which is much higher than in a farm environment. Hence, also in this case the sensitivity of the trial is too low.