Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC/2009_01045

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/CZ/2005/27 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/CZ/2005/27 was submitted by Monsanto on 10 November 2005 for the marketing (import and processing) of the insect-resistant and glyphosate-tolerant genetically modified maize MON88017 for food and feed uses under Regulation (EC) No. 1829/2003[1][2].

The application was officially acknowledged by EFSA on 11 January 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). Seven experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annexes I and II for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 4 April 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 21 April 2009 (The EFSA Journal, 2009, 1075, 1-28)[3], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 7 May 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.

[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] The same GM maize is the object of application GMO/CZ/2008/54 for authorisation of cultivation. The full environmental risk assessment is ongoing at the Biosafety Advisory Council and will be the subject of a separate advice.

[3] See: <http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902517555.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

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.

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the environment[4].

2. Molecular characterisation

According to the Biosafety Advisory Council the molecular characterisation data are considered as sufficient.

3. Food/feed safety assessment

3.1. Assessment of toxicity:

According to the Biosafety Advisory Council no major risks were identified concerning toxicity.

3.2. Assessment 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 influence the expression levels of maize proteins, producing potential allergens. Therefore, it might be relevant to analyze whether potential allergens do occur.

3.3. Nutritional assessment of the GM food/feed:

According to the Biosafety Advisory Council MON88017 is as nutritious as its non-GM counterpart and conventional maize varieties.

4. Monitoring

General surveillance is advised to follow-up unanticipated allergenicity aspects.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the notifier to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

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.

[4] 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.

In addition, the Biosafety Advisory Council recommends:

1) the introduction of of assessment of allergenicity of the whole GM crops in the frame of the revision of the EFSA guidance document on Food/Feed safety assessment;

2) general surveillance to follow up unanticipated allergenicity aspects.

p.o.

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/CZ/2005/27 *(ref: BAC_2007_PT_484)*
Annex II: Comments submitted on the EFSAnet (ref: BAC_2007_PT_485)

Wetenschappelijk Instituut Volksgezondheid | Institut Scientifique de Santé Publique
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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

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Conseil de Biosécurité

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Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT_484
Email. : bac@sbb.ihe.be

Comments of experts in charge of evaluating the application EFSA/GMO/CZ/2005/27

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 29 January 2007
Coordinator: Prof. dr. ir. Dirk Reheul (UGent)
Experts: Prof. Dr. Jacques Dommes (ULg), Dr. Ir. Leo Fiems (ILVO), Prof. Dr. Ir. Jean-Claude Grégoire (ULB), Prof. Robert Renaville (FUSAGx), Dr. Peter Smet (Consultant), Prof. Dr. Wim Stevens (UA), Mevr. Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: genetics, genetic engineering, general biochemistry, immunology, animal nutrition, alimentary allergology, analysis of food/feed, traceability of alimentary chain, toxicology, ecology, plant-insect relations, biodiversity, entomology, insect resistance, phytopathology, risk analysis, consumers info
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier EFSA/GMO/CZ/2005/27 concerns an application of the company Monsanto for the marketing of the genetically modified maize MON 88017 for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 January 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).

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Information provided is sufficient.

Comment 2

The fact that MON 88017 is similar to MON 863 in its protection against coleopteran pests, and the approval of MON 863 under Directive 2001/18/EC (Commission Decision, 2005b) and the fact that an approval under Regulation (EC) No. 258/97 is pending (Ref. No 6789-01-02-01), may be an advantage for the evaluation of MON 88017.

Comment 3

The modified maize has been presented as more resistant to glyphosate. What's the level of this resistance?
Because the modified maize is presented as more resistant to glyphosate, toxicity studies have to be realized to determine the residues level of glyphosate in MON88017, indeed more glyphosate would be applied on MON88017 that on normal maize.
As this GMO is more resistant it allows higher amounts of glyphosate to be used on crops, what about the persistence in the environment and/or contamination of groundwater ?
In this dossier, MON 88017 was often declared to be safe as the genes inserted are the same as the one of two other GMOs but some controversies has emerged about the safety of one of these (MON 863).
As MON 88017 would 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 theratogenic effects as well as 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 MON 88017 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 Monsanto can not assume that MON 88017 is safe because similar to wild type maize.

Additionnal comment from the coordinator: the comment above in italic is out of the scope of the application.

Comment 4

No comment/question

NB – My competence is in the environmental effects of GM plants; therefore my contribution in this dossier will be limited. Every time I will feel that the question asked is out of my field, I will use this "No comment/question" reply

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

Comments/Questions of the expert(s)

Comment 1

Information provided is sufficient.

Comment 2

No comments or questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Methods used for genetic modification, vector and inserted DNA fragments are well described.

Comment 2

No comments or questions

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

The traits introduced are well known and correctly described.

Comment 2

No comments or questions

Comment 3

No question or comments

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

Comments/Questions of the expert(s)

Comment 1

The number of insert integrations was evaluated by *Sca*I restriction of genomic DNA and hybridisation on southern blots. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data support the conclusion that this GMP contains a single integration site of the insert. Of course additional integrations of very small fragments of the insert cannot be excluded.
The number of copies inserted at this insertion site was evaluated through *Xba*I restriction, southern blotting and hybridisation. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data provided in the dossier support the conclusion that a single copy of the insert is integrated.
Mendelian segregation of the traits confirms that a single copy of the insert is present and that it is integrated into nuclear DNA.
Insert structure and intactness (both expression cassettes) were checked by hybridisation on southern blots of *Xho*I1 and/or *Hind*III restricted genomic DNA. Different probes covering the different parts of both expression cassettes were used. The data provided in the dossier support that MON88017 maize contains the expected full-length insert.
Absence of integration of vector backbone was checked through hybridisation on Southern blot. The blot was hybridised with a mixture of two probes spanning the entire length of the vector backbone. No integration of such vector sequence was detected. Of course integrations of very small fragments of the vector cannot be excluded.
In conclusion the data provided in the dossier support the following claims:

- MON88017 maize contains a single integration site of the DNA construct
- MON88017 maize contains a single copy of the DNA construct
- This insert in MON88017 maize is full length and show the expected structure
- No vector backbone is present in the genome of MON88017 maize.

Structure and intactness of insert was confirmed by PCR amplification of overlapping DNA fragments spanning the entire length of the insert. In addition these PCR fragments were cloned and sequenced. Compilation of sequences yielded the expected full-length sequence.
Sequencing was extended into neighbouring natural plant genomic DNA. A sequence of 878 bp was obtained upstream of the 5' side of the insert. A sequence of 1000 bp flanking the insert on its 3' side was obtained. These sequences corresponded to maize genomic DNA. PCR primers were designed in these flanking regions. They were used in PCR on genomic DNA from non-genetically modified maize. This yielded a 260 bp fragment. Sequencing data of this fragment suggests that integration of the insert was accompanied with limited modifications of the insertion site, i.e. a deletion of 25-27 bp and an addition of 20 bp. It is well known that T-DNA integration often induce this type of modifications.

Comment 2

No comments or questions

Comment 3

No question or comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Expression of the insert was evaluated through quantitative assays of the two protein products (Cry3Bb1 and CP4 EPSPS). This was done by ELISA on proteins extracted from whole plants or from specific plant organs. Plant material was collected at different growth stages at 3 locations in USA during the 2002 growing season. Additional plant material was harvested in Argentina during the 2003-2004 growing season. The results show that the Cry3Bb1 protein is expressed at different levels in all tested plant parts (leaf, pollen, silk, forage, forage root, grain, stover). The CPA EPSPS protein was also expressed in these plant parts (not tested in silk and stover). Such results were expected as constitutive promoters were used in the expression cassettes.
In addition possible expression of fusion proteins was considered. All possible reading frames at insert – genomic DNA junctions on both DNA strands were analysed. All possible peptides were FASTA aligned to different databases. No known immunological epitope was found.

Comment 2

No comments or questions

Comment 3

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

Comments/Questions of the expert(s)

Comment 1

No comments or questions

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

Comments/Questions of the expert(s)

Comment 1

Genetic stability of the insert was checked by southern analysis of *Xba*I-restricted genomic DNA. The blots were hybridised with a mixture of 4 DNA fragments spanning the entire length of the insert. This analysis was done over several generations (up to 7). The expected restriction fragments were always observed, suggesting that the insert was stably transmitted from generation to generation.

Comment 2

No comments or questions

Comment 3

SNPs 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 test 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

No comments or questions

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

According to EFSA (2005) the allergy risk evaluation of Cry3Bb1 protein in genetically modified maize MON 860 x MON 810 led to indirect evidence for an allergenicity risk for the protein being very low.

Comment 2

Differences in maize composition statistically significant 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.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Cry3Bb1 and CP4 EPSPS proteins used for the analysis of the allergenic effects, were produced by E. coli. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004).

Comment 2

No comments

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

The potential for toxicity of CP4 EPSPS and Cry3Bb1 proteins expressed in MON 88017 maize grain may be small, based on the low amount of CP4 EPSPS and Cry3Bb1 proteins found in maize grain, the absence of demonstrated acute toxicity to CP4 EPSPS and Cry3Bb1 in mice at doses greater than the range associated with proteins, the lack of sequence homology between known toxins and the CP4

EPSPS and Cry3Bb1 proteins, and the likelihood that the CP4 EPSPS and Cry3Bb1 proteins will be degraded in the gastrointestinal tract.

Comment 2

Toxicity tests reported in this dossier where done by Monsanto laboratories, what about independent labs toxicity results?

It is well-known that the pesticides are endocrinal disruptors. The new GMO has better a resistance to glyphosate. Moreover, Monsanto reported in this dossier that broilers fed with MON88017 have higher growth index which might be explained by a modification of endocrine axis. 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 are the first to be disrupted in illness so that they can not be removed from a toxicity study.

The toxicology effects are assumed to be negligible as the new OGM is constituted of the same inserted genes as MON 863 and NK603. In France, "la commission du genie biomoleculaire" has some doubts about the harmlessness of MON863 as there are significant differences in the pathology observed in rats after 90 days of alimentation with MON863.
Moreover, the authors indicate that "the Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of 99.8%, differing by only one of 653 amino acids. The single difference occurs at position 166. In MON 88017 and in the wild-type Cry3Bb1 protein, there is an aspartic acid at position 166. In MON 863, there is a glycine instead of an aspartic acid at this position. The physicochemical characterization and functional activity of the Cry3Bb1 protein produced in MON 88017 are equivalent to those of the Cry3Bb1 protein». Two protein even if similar are not equal so it might be that they have the same effects but the contrary is true as well. No assumption of the toxicity can be done on the bases of a similar protein. .
In conclusion, we require longer and more accurate toxicity studies to assess the harmlessness of this GMO.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Similar proteins to the two proteins present in MON 88017 maize have been assessed previously for safety (MON 863, NK603). Additionally, a battery of tests designed to evaluate the Cry3Bb1 variant protein and the native CP4 EPSPS protein present in MON 88017 maize for characteristics associated with food allergens and toxins raised no concern. The mature CP4 EPSPS in MON 88017 is identical to the bacterial enzyme of 455 amino acids and is targeted to the plant chloroplast. The Cry3Bb1 in MON 88017 differs from the native Cry3Bb1 by 6 amino acid changes, and differs from the in MON 863 variant by only 1 amino acid. Both novel proteins are expressed at relatively low levels in MON 88017.
CryBb1
No adverse effects were observed when Cry3Bb1 protein was ingested by mice at a dose of 1930 mg/kg bw. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known toxins and allergens. In vitro digestibility studies demonstrated that the Cry Bb1 variant was

rapidly degraded in simulated gastric fluid. Furthermore, the Cry Bb1 variant is not glycosylated in maize. Processing involving heat treatment rendered the CryBb1 variant protein non-functional.
The CryBb1 variant protein used in the studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, insecticidal activity and glycosylation. Both proteins were found to be equivalent.
CP4 EPSPS
In previous assessments (e.g. NK603), a battery of tests designed to evaluate the CP4 EPSPS protein for characteristics associated with food allergens and toxins raised no concern. The CP4 EPSPS protein shared no sequence homology with known toxins. There is a rapid digestion of the CP4 EPSPS protein in simulated digestive conditions, susceptibility to heating, and lack of acute toxicity for the CP4 EPSPS protein as determined by the mouse acute oral toxicity study.
The CP4 EPSPS protein used in these studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, glycosylation and functional activity. Both proteins were found to be equivalent.

Comment 2

Cry3Bb1

- The protein is rapidly and completely digested in simulated gastric fluid (SGF).
- The protein is digested in simulated intestinal fluid (SIF) with formation of fragments being active toxins (technical dossier pg 120 + fig 24). This seems to be part of its mode of action (English and Slatin (1992); Hofmann *et al.* (1988); Van Rie *et al.* (1989, 1990). These toxins bind to specific receptors on the brush border of the gut epithelium of rootworm larvae.
- **Question: Are there studies available which identify these receptors. If so, are these receptors also present in mammals?**
- On the other hand, this type of pesticide has a long history of safe use. Furthermore, toxicity studies indicate no adverse effect.
- Acute oral toxicity (mouse)

CP4 EPSPS

- The protein is rapidly and completely digested in SGF.
- Digestion in SIF seems to be much slower (Harrison *et al.* (1996)).
- **Remark: I disagree with the statement on pg 124 of the technical dossier, which says "… if any of the CP4 EPSPS protein did survive the gastric system, it would be rapidly degraded in the intestine". According to Harrison *et al.* (1996) 93-95% of added CP4 EPSPS was still present after a 10-min incubation in SIF. CP4 EPSPS activity had decreased to < 9% of the initial level after incubation of 285 min!**
- On the other hand, toxicity studies indicate no adverse effect.
- Acute oral toxicity (mouse)

Comment 3

No comments or questions

Comment 4

Cry3Bb1 has toxic effects on insect intestine. Monsanto did not give any scientific demonstration that this protein has no effects on human and animal intestine .
Monsanto based is safety assessment 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

No constituents other than the Cry3Bb1 and CP4 EPSPS proteins are novel. MON 88017 was shown to be compositionally equivalent to non-GM maize with comparable genetic background.

Comment 2

No comments or questions

Comment 3

As more glyphosate will be spread on cultures is is likely that more residues would be present on crops, what about glyphosate residues detected in MON88017?
What's the impact of these high glyphosate 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

Compositional studies were conducted to establish the nutritional adequacy of MON 88017 maize compared with a conventional control maize with similar genetic background, as well as with other commercially available maize hybrids. A reduction in approx. 23% in vitamin B1 levels was observed in MON 88017 grain samples compared with the conventional control maize (Vitamin B1 was consistently lower at each of the field sites). However, the levels were well within the 99% tolerance interval and well within the literature and historical range for maize grain. Other minor differences in fatty acid or amino acid constituents were not indicative of an overall pattern of change that could be attributed to the modification.
In conclusion, no particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.

Comment 2

No comments or questions

Comment 3

No questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

90-days feeding study in rats with MON 88017 maize grain.
The objective of these studies was to compare the responses of rats fed MON 88017 grain with the responses of rats fed the conventional control LH59 x LH198 that has background genetics similar to that of the MON 88017 grain (Kirkpatrick, 2005a), and compared with the responses of rats fed 6 commercial reference maize hybrids (Kirkpatrick, 2005b). All maize was grown in the same location at the same time (commercial reference hybrids on different fields). It is not mentioned in this study if MON 88017 maize was grown under glyphosate conditions. The study design included groups of Sprague-Dawley rats (20 rats/sex/group). One group was administered a diet containing 11% (w/w) MON 88017 supplemented with 22% (w/w) control grain. A second group was administered a diet containing 33% MON 88017. A third group was administered a diet containing 33% control grain. Another 6 groups were administered diets containing 33% reference maize varieties.
All animals survived, there were no test substance-related clinical observations. Body weights, food consumption and clinical pathology parameters were unaffected by the administration of MON 88017. No test-related effects were found on organ weights, and under macroscopic and microscopic examination. The few difference that were observed (higher mean food consumption and higher absolute neutrophil count in the 33% MON88017 females compared with the control group) fell within the range of responses of the six different groups fed conventional reference varieties of maize grain.

Poultry broilers feeding study with MON 88017 maize grain (42 days).
The study was undertaken to compare the wholesomeness of MON 88017 grain (treated with glyphosate herbicide? not mentioned in this study) to conventional control (LH59 x LH198) as well as to five commercial reference maize hybrids when fed to rapidly growing Ross x Ross 508 broilers (Taylor et al., 2005). Broilers were fed a starter diet (d0-21) and grower/finisher diet (d21-42) containing appr. 55% and 60% w/w maize, respectively, for all treatments. Treatments were randomly assigned to pens with five blocks for 16 pens (8 male, 8 female) with 10 broilers/pen for a total of 80 pens and 820 broilers. Broilers were weighed by pen on d0 and d42 and individually at study termination. Pen feed intake was determined at d42. At study termination, all surviving birds were processed to determine carcass yield and meat composition. Fat pad measurements were taken for each bird. One broiler/pen was randomly selected and sampled for breast and thigh meat quality assays.
Significant diet-by-gender interactions ($p<0.05$) were noted for live weight, final live body weight, chill weight, and thigh weight. No differences were observed in the percentage of moisture, protein, and fat in thigh meat and breast meat of broilers. Comparison of the MON 88017 fed birds to the population of the other diets fed showed no differences on all performance parameters, carcass yields, or meat quality parameters measured.

In conclusion, the results of the broiler feeding study showed that there were no biologically significant differences on the parameters tested between broilers fed MON 88017 or the broilers fed control maize. Minor differences noted were consistent with literature values and within natural variability.

In conclusion (and as concluded by the applicant), these studies confirm the absence of any toxic effects associated to the introduced proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modification. In conclusion, there was no evidence of any adverse effects on human or animal health.

Comment 2

Toxicity studies indicate no adverse effects.
- 13-week feeding study in the rat (testing of whole food)
- 42-day feeding study in broiler chickens (testing of whole feed)

Conclusion: The toxicological data show no adverse effects after administration of either protein as such, nor as whole food. As a result, the use of the genetically modified **maize MON 88017** can be regarded as safe for animals and human beings.

Comment 3

No comments or questions

Comment 4

Comparison with known toxic and allergens is not sufficient to assess harmlessness as the protein introduced in maize are not present in other food usually consumed by humans and animals. Moreover chronic toxicity has been demonstrated for MON 863.
It is also required to assess harmlessness of the newly expressed proteins on intestinal epithelium as this tissue is the target of the protein even if in an other species.
Subchronic study demonstrated that there is a significant increase in neutrophil count in males which is not justified in a scientific manner. Confrontation of data with data of other study is not valid. There is a lack of a longer chronic study in other to assess effects of long term ingestion of MON88017

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Maize itself (Zea mais) rarely induces allergic reactions in man as a food nor as a pollination plant (heavy pollen)

The new proteins Cry3Bb1 and CP4 EPSPS were already evaluated for allergenicity in the context of MON 863 and NK603 maize.
The risk for allergenicity can be assessed by combining different approaches (Helm 2003):
- content of the protein(s) in the food/feed

- digestibility of the protein(s) and stability in acid proteases in the food/feed
- comparison of the amino acid structure of the protein(s) with known allergens
- testing with specific IgE from allergic patients
- testing in animal models

For three of these parameters the proteins Cry3Bb1 and CP4 EPSPS showed a good profile:
- low content of proteins Cry3Bb1 and CP4 EPSPS in the maize end product
- good digestibility in acid peptic digestion

It has to be mentioned nevertheless that not all allergens are stable proteins (eg Mal d 1 from apple) (Ebo et al. 2005)

As far as the comparison of the proteins Cry3Bb1 and CP4 EPSPS with known allergen structures is concerned:

- protein Cry3Bb1 showed some similarity with the Anisakis simplex tropomyosin Ani s3. The overlap of 120 aa contained four gaps and showed 27.5 % identity with an E score of 1.1. The longest stretch of continuous aa was 3; this was considered as non significant. Follow up of this situation is advised since tropomyosin are to be considered as pan-allergen in a high number of living animal, with possible cross reactivity (Ebo and Stevens 2001).

- protein CP4 EPSPS had an alignment of 30.5 % identity with Dermatophagoides farinae Der f 2over 82 aa with a high E score of 0.41. The longest stretch of contiguous aa was 5. This similarity was evaluated as insignificant. Follow up of this situation is advised since Dermatophagoides sp belong to the most frequently occurring inhalation allergens in moderate climate zones such as in important parts of the US and Europe.

Testing with specific IgE or animal studies were not done (not relevant at this moment).

The author also searched medical databases in order to find reports on allergenicity of the proteins Cry3Bb1 and CP4 EPSPS. No relevant data were found.
In conclusion, it can be stated that at present there is no evidence that the GM maize containing the proteins Cry3Bb1 and CP4 EPSPS will induce allergic reactions. Continuous surveillance is advised. It has also to be taken in consideration that other forms of allergic reactions than IgE mediated are possible (Bernstein et al. 2003)

Comment 2

MON 88017 maize contains 2 new proteins with distinct properties. The toxic and allergenic effects of both proteins were individually discussed. The applicant believes that the general surveillance plan endorsed by EFSA for NK603 can also serve as a model for MON 88017. However, it is not sufficiently stated that there is no synergism between both proteins with regard to possible detrimental effects. On P.109, Part I of the Technical Dossier, it is stated that these proteins are similar to the proteins expressed in MON 863 and NK603, respectively, that have been considered safe by EFSA. This is not in agreement with the draft report of the EFSA (2006) “Safety and Nutritional Assessment of GM Plant derived Foods/Feed The role of animal feeding trials”, where it is emphasized that a safety assessment of a novel food/feed should be based on a case by case approach. Obviously, this is

not the case in this dossier. Furthermore, Monsanto has not done any effort to isolate sufficient Cry3Bb1 and CP4 EPSPS proteins from MON 88017 maize, but they used Cry3Bb1 and CP4 EPSPS proteins produced by E. coli (P.116, Part I of the Technical Dossier). It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004). Monsanto used simulated gastric and intestinal fluids to test the digestion of Cry3Bb1 and CP4 EPSPS proteins. It has been shown that a rapid in vivo degradation of Cry proteins (Cry1Ab) does not always occur (Chowdhury et al., 2003). Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered suitable tools to address the allergenic potential of a protein.

Comment 3

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

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

There are no indications suggesting nutritional inconveniences in comparison to conventional maize varieties. It was concluded from the animal performance in broiler studies that there was a nutritional equivalence compared with conventional control lines (Taylor et al., 2005).

The applicant only discusses MON 88017 in this dossier. What effects can be expected if this novel food/feed is used in diets containing other GM food/feed, such as soy beans, rape seed, rice, …?

The effect of a combined use of MON 88017 with other novel foods/feeds in diets for animals and humans is not extensively investigated. Are interactions between proteins from MON 88017 and proteins from other GM plants excluded?

Comment 2

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

No comments or questions

Comment 2

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

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

Comments/Questions of the expert(s)

Comment 1

Adequately examined and described.

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

No comments or questions.

Comment 2

The documentation is satisfactory.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

The question is not relevant here, as developed in the technical dossier.

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.

Comment 2

The probability that (spillage + establishment + contamination) is limited at some parts of the itinerary (e g at ports), but not necessarily along the transportation routes. Even though it can not survive the winter, maize from spilled seeds can develop one generation on the sites of spilling, leading to potential dissemination of spores. 1% of the pollen beyond 50 m (Sears and Stanley-Horn, 2000) does not seem negligible to me. If we do not know the routes, we do not know if maize is grown along the roads
I feel that more specific details are needed regarding the packing and other means of confinement during transportation and storage

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

The documentation is satisfactory.

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

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

Effects on hormonal status of animals must be determined.
How was the specificity of MON 88017 Cry3Bb1 demonstrated in animals, including mammals, birds, fish and non-target arthropods. Was it tested on all these animals?

Additionnal comment from the coordinator: the comment above is out of the scope of the application.

Comment 3

The documentation is satisfactory.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

See comment on D.7.9.

Comment 2

Monsanto should provide more accurate toxicity studies in order to demonstrate its hypothesis of no human toxicity.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

Studies of Taylor et al. (2005) indicated that broiler mortality based on diets containing MON 88017 fell within the range reported for commercial maize varieties. See also comment on D.7.9.

Comment 2

As for human, there is a need of more accurate toxicity studies.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

This question is not relevant in view of the import of maize MON 88017 into the EU.

Comment 2

No comments

Comment 3

Irrelevant here.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No comments or questions

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No comments or questions

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No comments or questions

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No comments or questions

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

Comments/Questions of the expert(s)

Comment 1

No comments or questions

Comment 2

I have not seen any risk assessment regarding changes in agricultural practices, even though it is of wide concern that GM spore dissemination might jeopardize organic agriculture. Since I am not totally convinced by the alleged low risk for genetic contamination, I cannot exclude this risk to other forms of agriculture.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No comments or questions

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Bioveiligheidsraad
Conseil de Biosécurité

05-04-2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT_485
Email. : bac@sbb.ihe.be

Application EFSA/GMO/CZ/2005/27
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 January 2007
Coordinator: Prof. dr. ir. Dirk Reheul (UGent)
Experts: Prof. Dr. Jacques Dommes (ULg), Dr. Ir. Leo Fiems (ILVO), Prof. Dr. Ir. Jean-Claude Grégoire (ULB), Prof. Robert Renaville (FUSAGx), Dr. Peter Smet (Consultant), Prof. Dr. Wim Stevens (UA), Mevr. Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: genetics, genetic engineering, general biochemistry, immunology, animal nutrition, alimentary allergology, analysis of food/feed, traceability of alimentary chain, toxicology, ecology, plant-insect relations, biodiversity, entomology, insect resistance, phytopathology, risk analysis, consumers info
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier EFSA/GMO/CZ/2005/27 concerns an application of the company Monsanto for the marketing of the genetically modified maize MON 88017 for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 January 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)

List of comments submitted on the EFSAnet

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).
When needed, comments from the experts have been summarized by the coordinator to fit within the 4000 characters limit imposed by the EFSAnet.
For the full comments of the Belgian experts and the bibliographic references we refer to the document given in annex 2. It displays all the comments as there were transmitted by the experts (ref. BAC_2007_PT_484)

A. GENERAL INFORMATION

Comments submitted on the EFSAnet

The modified maize has been presented as more resistant to glyphosate. What's the level of this resistance?
Because the modified maize is presented as more resistant to glyphosate, toxicity studies have to be realized to determine the residues level of glyphosate in MON88017, indeed more glyphosate would be applied on MON88017 that on normal maize.

In this dossier, MON 88017 was often declared to be safe as the genes inserted are the same as the one of two other GMOs but some controversies has emerged about the safety of one of these (MON 863).
As MON 88017 would 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 theratogenic effects as well as 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 MON 88017 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 Monsanto can not assume that MON 88017 is safe because similar to wild type maize.

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

Comments submitted on the EFSAnet

None

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments submitted on the EFSAnet

Methods used for genetic modification, vector and inserted DNA fragments are well described.

D. INFORMATION RELATING TO THE GM PLANT

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

Comments submitted on the EFSAnet

The traits introduced are well known and correctly described.

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

Comments submitted on the EFSAnet

The number of insert integrations was evaluated by *Sca*I restriction of genomic DNA and hybridisation on southern blots. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data support the conclusion that this GMP contains a single integration site of the insert. Of course additional integrations of very small fragments of the insert cannot be excluded.
The number of copies inserted at this insertion site was evaluated through *Xba*I restriction, southern blotting and hybridisation. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data provided in the dossier support the conclusion that a single copy of the insert is integrated.
Mendelian segregation of the traits confirms that a single copy of the insert is present and that it is integrated into nuclear DNA.
Insert structure and intactness (both expression cassettes) were checked by hybridisation on southern blots of *Xho*I1 and/or *Hind*III restricted genomic DNA. Different probes covering the different parts of both expression cassettes were used. The data provided in the dossier support that MON88017 maize contains the expected full-length insert.
Absence of integration of vector backbone was checked through hybridisation on Southern blot. The blot was hybridised with a mixture of two probes spanning the entire length of the vector backbone. No integration of such vector sequence was detected. Of course integrations of very small fragments of the vector cannot be excluded.
In conclusion the data provided in the dossier support the following claims:

- MON88017 maize contains a single integration site of the DNA construct
- MON88017 maize contains a single copy of the DNA construct
- This insert in MON88017 maize is full length and show the expected structure
- No vector backbone is present in the genome of MON88017 maize.

Structure and intactness of insert was confirmed by PCR amplification of overlapping DNA fragments spanning the entire length of the insert. In addition these PCR fragments were cloned and sequenced. Compilation of sequences yielded the expected full-length sequence.
Sequencing was extended into neighbouring natural plant genomic DNA. A sequence of 878 bp was obtained upstream of the 5' side of the insert. A sequence of 1000 bp flanking the insert on its 3' side was obtained. These sequences corresponded to maize genomic DNA. PCR primers were designed in these flanking regions. They were used in PCR on genomic DNA from non-genetically modified maize. This yielded a 260 bp fragment. Sequencing data of this fragment suggests that integration of the insert was accompanied with limited modifications of the insertion site, i.e. a deletion of 25-27 bp

and an addition of 20 bp. It is well known that T-DNA integration often induce this type of modifications.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments submitted on the EFSAnet

1. Expression of the insert was evaluated through quantitative assays of the two protein products (Cry3Bb1 and CP4 EPSPS). This was done by ELISA on proteins extracted from whole plants or from specific plant organs. Plant material was collected at different growth stages at 3 locations in USA during the 2002 growing season. Additional plant material was harvested in Argentina during the 2003-2004 growing season. The results show that the Cry3Bb1 protein is expressed at different levels in all tested plant parts (leaf, pollen, silk, forage, forage root, grain, stover). The CPA EPSPS protein was also expressed in these plant parts (not tested in silk and stover). Such results were expected as constitutive promoters were used in the expression cassettes.
In addition possible expression of fusion proteins was considered. All possible reading frames at insert – genomic DNA junctions on both DNA strands were analysed. All possible peptides were FASTA aligned to different databases. No known immunological epitope was found.

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

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

1. Genetic stability of the insert was checked by southern analysis of *Xba*I-restricted genomic DNA. The blots were hybridised with a mixture of 4 DNA fragments spanning the entire length of the insert. This analysis was done over several generations (up to 7). The expected restriction fragments were always observed, suggesting that the insert was stably transmitted from generation to generation.

2. SNPs 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 test 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 submitted on the EFSAnet

None

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 submitted on the EFSAnet

Differences in maize composition statistically significant 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.7.2 Production of material for comparative assessment

Comments submitted on the EFSAnet

None

D.7.3 Selection of material and compounds for analysis

Comments submitted on the EFSAnet

Cry3Bb1 and CP4 EPSPS proteins used for the analysis of the allergenic effects, were produced by E. coli. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004).

D.7.4 Agronomic traits

None

D.7.5 Product specification

Comments submitted on the EFSAnet

None

D.7.6 Effect of processing

None

D.7.7 Anticipated intake/extent of use

Comments submitted on the EFSAnet

None

D.7.8 Toxicology

Comments submitted on the EFSAnet

1. Toxicity tests reported in this dossier where done by Monsanto laboratories, what about independent labs toxicity results?

2. The potential for toxicity of CP4 EPSPS and Cry3Bb1 proteins expressed in MON 88017 maize grain may be small, based on the low amount of CP4 EPSPS and Cry3Bb1 proteins found in maize grain, the absence of demonstrated acute toxicity to CP4 EPSPS and Cry3Bb1 in mice at doses greater than the range associated with proteins, the lack of sequence homology between known toxins and the CP4 EPSPS and Cry3Bb1 proteins.

3. It is well-known that the pesticides are endocrinal disruptors. Monsanto reported in this dossier that broilers fed with MON88017 have higher growth index which might be explained by a modification of endocrine axis. 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 are the first to be disrupted in illness so that they can not be removed from a toxicity study.

The toxicology effects are assumed to be negligible as the new OGM is constituted of the same inserted genes as MON 863 and NK603. In France, “la commission du genie biomoleculaire” has some doubts about the harmlessness of MON863 as there are significant differences in the pathology observed in rats after 90 days of alimentation with MON863.
Moreover, the authors indicate that “the Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of 99.8%, differing by only one of 653 amino acids. The single difference occurs at position 166. In MON 88017 and in the wild-type Cry3Bb1 protein, there is an aspartic acid at position 166. In MON 863, there is a glycine instead of an aspartic acid at this position. The physicochemical characterization and functional activity of the Cry3Bb1 protein produced in MON 88017 are equivalent to those of the Cry3Bb1 protein». Two protein even if similar are not equal so it might be that they have the same effects but the contrary is true as well. No assumption of the toxicity can be done on the bases of a similar protein. .
In conclusion, longer and more accurate toxicity studies are required to assess the harmlessness of this GMO.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments submitted on the EFSAnet

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

Similar proteins to the two proteins present in MON 88017 maize have been assessed previously for safety (MON 863, NK603). Additionally, a battery of tests designed to evaluate the Cry3Bb1 variant protein and the native CP4 EPSPS protein present in MON 88017 maize for characteristics associated with food allergens and toxins raised no concern. The mature CP4 EPSPS in MON 88017 is identical to the bacterial enzyme of 455 amino acids and is targeted to the plant chloroplast. The Cry3Bb1 in MON 88017 differs from the native Cry3Bb1 by 6 amino acid changes, and differs from the in MON 863 variant by only 1 amino acid. Both novel proteins are expressed at relatively low levels in MON 88017.

CryBb1

No adverse effects were observed when Cry3Bb1 protein was ingested by mice at a dose of 1930 mg/kg bw. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known toxins and allergens. In vitro digestibility studies demonstrated that the Cry Bb1 variant was rapidly degraded in simulated gastric fluid. Furthermore, the Cry Bb1 variant is not glycosylated in maize. Processing involving heat treatment rendered the CryBb1 variant protein non-functional.
The CryBb1 variant protein used in the studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, insecticidal activity and glycosylation. Both proteins were found to be equivalent.

The protein is rapidly and completely digested in simulated gastric fluid (SGF).
The protein is digested in simulated intestinal fluid (SIF) with formation of fragments being active toxins (technical dossier pg 120 + fig 24). This seems to be part of its mode of action (English and Slatin (1992); Hofmann *et al.* (1988); Van Rie *et al.* (1989, 1990). These toxins bind to specific receptors on the brush border of the gut epithelium of rootworm larvae.
Question: Are there studies available which identify these receptors. If so, are these receptors also present in mammals?

Acute oral toxicity (mouse)

CP4 EPSPS

In previous assessments (e.g. NK603), a battery of tests designed to evaluate the CP4 EPSPS protein for characteristics associated with food allergens and toxins raised no concern. The CP4 EPSPS protein shared no sequence homology with known toxins. There is a rapid digestion of the CP4 EPSPS protein in simulated digestive conditions, susceptibility to heating, and lack of acute toxicity for the CP4 EPSPS protein as determined by the mouse acute oral toxicity study.
The CP4 EPSPS protein used in these studies was obtained in an *E. coli* production system. The equivalency of the MON 88017 maize produced protein to the *E. coli*- produced protein was evaluated by comparing the molecular weight, immunological reactivity, glycosylation and functional activity. Both proteins were found to be equivalent.

The protein is rapidly and completely digested in SGF.
Digestion in SIF seems to be much slower (Harrison *et al.* (1996)).
Remark: we disagree with the statement on pg 124 of the technical dossier, which says "… if any of the CP4 EPSPS protein did survive the gastric system, it would be rapidly degraded in the intestine". According to Harrison *et al.* (1996) 93-95% of added CP4 EPSPS was still present

after a 10-min incubation in SIF. CP4 EPSPS activity had decreased to < 9% of the initial level after incubation of 285 min!

Acute oral toxicity (mouse)

D.7.8.2 Testing of new constituents other than proteins

Comments submitted on the EFSAnet

None

D.7.8.3 Information on natural food and feed constituents

Comments submitted on the EFSAnet

Compositional studies were conducted to establish the nutritional adequacy of MON 88017 maize compared with a conventional control maize with similar genetic background, as well as with other commercially available maize hybrids. A reduction in approx. 23% in vitamin B1 levels was observed in MON 88017 grain samples compared with the conventional control maize (Vitamin B1 was consistently lower at each of the field sites). However, the levels were well within the 99% tolerance interval and well within the literature and historical range for maize grain. Other minor differences in fatty acid or amino acid constituents were not indicative of an overall pattern of change that could be attributed to the modification.
In conclusion, no particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.

D.7.8.4 Testing of the whole GM food/feed

Comments submitted on the EFSAnet

1. 90-days feeding study in rats with MON 88017 maize grain.
The objective of these studies was to compare the responses of rats fed MON 88017 grain with the responses of rats fed the conventional control LH59 x LH198 that has background genetics similar to that of the MON 88017 grain (Kirkpatrick, 2005a), and compared with the responses of rats fed 6 commercial reference maize hybrids (Kirkpatrick, 2005b). All maize was grown in the same location at the same time (commercial reference hybrids on different fields). It is not mentioned in this study if MON 88017 maize was grown under glyphosate conditions. The study design included groups of Sprague-Dawley rats (20 rats/sex/group). One group was administered a diet containing 11% (w/w) MON 88017 supplemented with 22% (w/w) control grain. A second group was administered a diet containing 33% MON 88017. A third group was administered a diet containing 33% control grain. Another 6 groups were administered diets containing 33% reference maize varieties.
All animals survived, there were no test substance-related clinical observations. Body weights, food consumption and clinical pathology parameters were unaffected by the administration of MON 88017. No test-related effects were found on organ weights, and under macroscopic and microscopic examination. The few difference that were observed (higher mean food consumption and higher absolute neutrophil count in the 33% MON88017 females compared with the control group) fell within the range of responses of the six different groups fed conventional reference varieties of maize grain.

2. Poultry broilers feeding study with MON 88017 maize grain (42 days).
The study was undertaken to compare the wholesomeness of MON 88017 grain (treated with glyphosate herbicide? not mentioned in this study) to conventional control (LH59 x LH198) as well as to five commercial reference maize hybrids when fed to rapidly growing Ross x Ross 508 broilers (Taylor et al., 2005). Broilers were fed a starter diet (d0-21) and grower/finisher diet (d21-42) containing appr. 55% and 60% w/w maize, respectively, for all treatments. Treatments were randomly assigned to pens with five blocks for 16 pens (8 male, 8 female) with 10 broilers/pen for a total of 80 pens and 820 broilers. Broilers were weighed by pen on d0 and d42 and individually at study termination. Pen feed intake was determined at d42. At study termination, all surviving birds were processed to determine carcass yield and meat composition. Fat pad measurements were taken for each bird. One broiler/pen was randomly selected and sampled for breast and thigh meat quality assays.
Significant diet-by-gender interactions ($p<0.05$) were noted for live weight, final live body weight, chill weight, and thigh weight. No differences were observed in the percentage of moisture, protein, and fat in thigh meat and breast meat of broilers. Comparison of the MON 88017 fed birds to the population of the other diets fed showed no differences on all performance parameters, carcass yields, or meat quality parameters measured.
In conclusion, the results of the broiler feeding study showed that there were no biologically significant differences on the parameters tested between broilers fed MON 88017 or the broilers fed control maize. Minor differences noted were consistent with literature values and within natural variability.

In conclusion (and as concluded by the applicant), these studies confirm the absence of any toxic effects associated to the introduced proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modification. In conclusion, there was no evidence of any adverse effects on human or animal health.

3. Chronic toxicity has been demonstrated for MON 863.
Subchronic study demonstrated that there is a significant increase in neutrophil count in some groups of females which is not justified in a scientific manner. Confrontation of data with data of other studies is not valid. There is a lack of a longer chronic study in other to assess effects of long term ingestion of MON88017.

D.7.9 Allergenicity

Comments submitted on the EFSAnet

1. Maize itself (Zea mais) rarely induces allergic reactions in man as a food nor as a pollination plant
The new proteins Cry3Bb1 and CP4 EPSPS were already evaluated for allergenicity in the context of MON 863 and NK603 maize.
The risk for allergenicity can be assessed by combining different approaches (Helm 2003):

- content of the protein(s) in the food/feed
- digestibility of the protein(s) and stability in acid proteases in the food/feed
- comparison of the amino acid structure of the protein(s) with known allergens
- testing with specific IgE from allergic patients
- testing in animal models

For three of these parameters the proteins Cry3Bb1 and CP4 EPSPS showed a good profile:

- low content of proteins Cry3Bb1 and CP4 EPSPS in the maize end product
- good digestibility in acid peptic digestion

It has to be mentioned nevertheless that not all allergens are stable proteins (eg Mal d 1 from apple) (Ebo et al. 2005)

As far as the comparison of the proteins Cry3Bb1 and CP4 EPSPS with known allergen structures is concerned:

- protein Cry3Bb1 showed some similarity with the Anisakis simplex tropomyosin Ani s3. The overlap of 120 aa contained four gaps and showed 27.5 % identity with an E score of 1.1. The longest stretch of continuous aa was 3; this was considered as non significant. Follow up of this situation is advised since tropomyosin are to be considered as pan-allergen in a high number of living animal, with possible cross reactivity (Ebo and Stevens 2001).
- protein CP4 EPSPS had an alignment of 30.5 % identity with Dermatophagoides farinae Der f 2over 82 aa with a high E score of 0.41. The longest stretch of contiguous aa was 5. This similarity was evaluated as insignificant. Follow up of this situation is advised since Dermatophagoides sp belong to the most frequently occurring inhalation allergens in moderate climate zones such as in important parts of the US and Europe.

No reports in medical databases were found on allergenicity of the proteins Cry3Bb1 and CP4 EPSPS. Continuous surveillance is advised. It has also to be taken in consideration that other forms of allergic reactions than IgE mediated are possible (Bernstein et al. 2003)

2. MON 88017 maize contains 2 new proteins with distinct properties. The toxic and allergenic effects of both proteins were individually discussed. The applicant believes that the general surveillance plan endorsed by EFSA for NK603 can also serve as a model for MON 88017. However, it is not sufficiently stated that there is no synergism between both proteins with regard to possible detrimental effects. On P.109, Part I of the Technical Dossier, it is stated that these proteins are similar to the proteins expressed in MON 863 and NK603, respectively, that have been considered safe by EFSA. This is not in agreement with the draft report of the EFSA (2006) “Safety and Nutritional Assessment of GM Plant derived Foods/Feed The role of animal feeding trials”, where it is emphasized that a safety assessment of a novel food/feed should be based on a case by case approach. Obviously, this is not the case in this dossier. Furthermore, Monsanto has not done any effort to isolate sufficient Cry3Bb1 and CP4 EPSPS proteins from MON 88017 maize, but they used Cry3Bb1 and CP4 EPSPS proteins produced by E. coli (P.116, Part I of the Technical Dossier). It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004). Monsanto used simulated gastric and intestinal fluids to test the digestion of Cry3Bb1 and CP4 EPSPS proteins. It has been shown that a rapid in vivo degradation of Cry proteins (Cry1Ab) does not always occur (Chowdhury et al., 2003). Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered suitable tools to address the allergenic potential of a protein.

3. Monsanto claims no allergenicity for the new proteins because they don’t share aminoacids sequences with known allergens but again these proteins are new in human alimentation and so there is a need of specific scientific studies.

D.7.10 Nutritional assessment of GM food/feed

Comments submitted on the EFSAnet

There are no indications suggesting nutritional inconveniences in comparison to conventional maize varieties. It was concluded from the animal performance in broiler studies that there was a nutritional equivalence compared with conventional control lines (Taylor et al., 2005).

The applicant only discusses MON 88017 in this dossier. What effects can be expected if this novel food/feed is used in diets containing other GM food/feed, such as soy beans, rape seed, rice, …?

The effect of a combined use of MON 88017 with other novel foods/feeds in diets for animals and humans is not extensively investigated. Are interactions between proteins from MON 88017 and proteins from other GM plants excluded?

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

Comments submitted on the EFSAnet

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

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

Comments submitted on the EFSAnet

None

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 submitted on the EFSAnet

None

D.9.2 Selective advantage or disadvantage

Comments submitted on the EFSAnet

None

D.9.3 Potential for gene transfer

Comments submitted on the EFSAnet

The probability that (spillage + establishment + contamination) is limited at some parts of the itinerary (e g at ports), but not necessarily along the transportation routes. Even though it can not survive the winter, maize from spilled seeds can develop one generation on the sites of spilling, leading to potential dissemination of pollen. 1% of the pollen beyond 50 m (Sears and Stanley-Horn, 2000) does not seem negligible to me. If we do not know the routes, we do not know if maize is grown along the roads
I feel that more specific details are needed regarding the packing and other means of confinement during transportation and storage

D.9.4 Interactions between the GM plant and target organism

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

D.9.6 Effects on human health

Monsanto should provide more accurate toxicity studies in order to demonstrate its hypothesis of no human toxicity.

D.9.7 Effects on animal health

Studies of Taylor et al. (2005) indicated that broiler mortality based on diets containing MON 88017 fell within the range reported for commercial maize varieties. See also comment on D.7.9.

D.9.8 Effects on biogeochemical processes

Comments submitted on the EFSAnet

None

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

Comments submitted on the EFSAnet

None

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments submitted on the EFSAnet

None

D.11. ENVIRONMENTAL MONITORING PLAN

None