Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC/2009_01368

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-1507 from Pioneer Hi-Bred under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-1507 was submitted by Pioneer Hi-Bred International, Inc./ Mycogen Seeds on 29 June 2007 for renewal of authorisation of the insect resistant and glufosinate tolerant genetically modified (GM) maize 1507 for feed applications (feed materials and feed additives) according to Article 20 of Regulation (EC) No. 1829/2003[1].

Maize 1507 has already been subject previously to several notifications:
- For the placing on the market as food or food ingredient under Regulation (EC) No 1829/2003. Approved by Commission Decision (2006/197/EC)[2] of 3 March 2006;
- For the placing on the market for import and processing of feed (notification C/NL/00/10 submitted under Directive 2001/18/EC); Approved by Commission Decision 2005/772/EC of 3 November 2005[3];
- For the placing on the market for import, feed, industrial processing and cultivation (notification C/ES/01/01 submitted under Directive 2001/18/EC); the authorization procedure is still running. Belgium has previously issued a scientific opinion related to this notification (report of 18 August 2006 of the Division of Biosafety and Biotechnology on mandate of the Biosafety Advisory Council).

Additionally, maize 1507 has been entered on the community register of GM Food and Feed as an existing product under Article 20 of Regulation (EC) No 1829/2003.

The application EFSA/GMO/RX-1507 was officially acknowledged by EFSA on 15 April 2008. On the same date EFSA started the formal three-month 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).

[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] Commission Decision 2006/197/EC of 3 March 2006 authorising the placing on the market of food containing, consisting of, or produced from genetically modified maize line 1507 (DAS-Ø15Ø7-1) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (OJ L 70, 09.03.2006, p. 82)
[3] Commission Decision 2005/772/EC of 3 November 2005 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (Zea mays L., line 1507) genetically modified for resistance to certain lepidopteran pests and for tolerance to the herbicide glufosinate-ammonium (OJ L 291, 05.11.2005, p. 42)

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 chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB) to evaluate the dossier. Three experts answered positively to this request and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 4 July 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 28 May 2009 (The EFSA Journal, 2009, 1138, 1-11)[4], and published together with the responses of the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 19 June 2009 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

Scientific evaluation

1. Environmental risk assessment

The scope of this application is for feed materials and feed additives which are produced from GM maize 1507 and only includes products which contain no viable plant parts. Therefore, there are no requirements to perform an environmental risk assessment.

2. Molecular characterisation

With regard to the molecular characterisation, the Belgian experts are of the opinion that information received is sufficient.

3. Feed safety assessment

3.1. Assessment of toxicity

Although this dossier is a renewal of maize 1507 the dossier refers to several studies reporting trials regarding feed safety assessment. A number of these trials lack scientific strength. The studies of Kuhn (1998) and Brooks (2000) with mice provide data only from one treatment, so that a comparison with a control group is not possible. The study reported by MacKenzie (2003) has not sufficient statistical power, since 63 animals per treatment are necessary instead of 12 to find a statistically significant difference, based on the method presented by Berndtson (1991).

3.2. Nutritional value

The study of Zeph (2000) with broilers does not provide information on the variability within treatments, so that the power of the statistical method cannot be calculated. Moreover, overall mortality rate and feed conversion are rather high, while growth rate is rather low.

[4] See: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902573013.htm

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 no major risks concerning the environment were identified.

The lack of quality of animal trials for toxicity testing and testing of the nutritional value provided by the applicant for the renewal urges the Biosafety Advisory Council not to draw conclusions about the feed safety of this GM maize.

p.o.

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

Annex: Full comments of experts in charge of evaluating application EFSA/GMO/RX-1507 *and comments submitted on the EFSAnet (ref: BAC_2008_786)*

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04/07/2008

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-1507
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 21 May 2008
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Jacques Dommes (ULg), Rony Geers (KUL), Peter Smet (Consultant)
Domains of expertise of experts involved: Genetics, genetic engineering, molecular characterisation, transgene expression, animal nutrition, statistics, toxicology
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/RX-1507** concerns an application of the company **Pioneer** for the renewal of authorisation of the genetically modified **maize 1507** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 15 April 2008.
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) toxicity and/or 3) feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

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)

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

Comments/Questions of the expert(s)

Comment 1

I do not agree with the statement on p. 19 of part 1 "This fact on the complexity of maize genome would made it very difficult to determine by PCR analysis whether the 5' and 3' flanking genomic sequences are in fact continuous in the untransformed maize". I agree that retrotransposons are a natural source of genetic variation, but the LTR-like sequence located at the 5' border does not seem to be included in a functional mobile genetic element. In addition phenotypic stability and presence of insert were confirmed over several generations, suggesting no remodeling of the insert and neighbouring DNA. It is not clear to me whether the applicant did actually try to determine if the 5' and

3' flanking genomic sequences are continuous in the untransformed maize. This can be done by PCR using primers hybridising in region 1 and 15 (fig. 15). This data would be useful to assess any unforeseen effect linked to gene disruption or modification of the flanking genomic DNA.

Nor do I agree with the third paragraph on p. 19: it is very unlikely that natural cross-over recombination would occur between linked genomic sequences. However this remark is not relevant for the safety evaluation of this maize.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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)

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

D.7.5 Product specification

Comments/Questions of the expert(s)

D.7.6 Effect of processing

Comments/Questions of the expert(s)

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

The studies of Kuhn (1998) and Brooks (2007) with mice provide data only from one treatment, so that a comparison with a control group is not possible. The study reported by MacKenzie (2003) has not sufficient statistical power, since 63 animals per treatment are necessary in stead of 12 to find a statistically significant difference, based on the method presented by Berndtson (1991).

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

a) Degradation of the cry1F protein in simulated gastric fluid (Schafer and Korjagin, 2002).

Test protein (and its minor degradation fragments) were not detectable at 15 seconds as demonstrated by both SDS-PAGE and Western blot analysis.
Remark: figure 6, panel A (Western blot) is of no use, due to the bad quality.

7b) Degradation of the cry1F protein in simulated intestinal fluid (EFSA, 2004).

In simulated intestinal fluid (pancreatin), the trypsin-resistant CRY1F core protein proved stable over the entire exposure of 120 minutes.

7c) cry1F: Acute Oral Toxicity Study in Mice (Kuhn, 1998; MacKenzie, 2007).

The test substance, Cry1F *bacillus thuringiensis subsp. Aizawai* Delta-endotoxin, was evaluated for its oral toxicity potential in albino mice when administered as a gavage dose at a level of 5050 mg/kg to males and females. The test substance was administered as a 15% w/v concentration in 2% w/v aqueous carboxymethyl cellulose (CMC).
No mortality occurred during the study. There were no clinical signs of toxicity exhibited at any time throughout the study. There was no meaningful effect on body weight gain. The gross necropsy conducted at termination revealed no observable abnormalities. The acute oral LD_{50} was determined to be greater than 5050 mg/kg.

7d) Degradation of the PAT protein in simulated gastric fluid (OECD, 1999).

The protein is rapidly degraded.

7e) Degradation of the PAT protein in simulated intestinal fluid ().

Test not performed. No data provided.

7f) PAT: Acute Oral Toxicity Study in Mice (Brooks, 2000).

PAT Microbial protein, which was 84% pure microbial protein, was evaluated for acute toxicity. Five male and five female CD-1 mice received 6000 mg/kg of the test material (containing appr. 5000 mg/kg PAT) as a 25% w/v suspension in aqueous 0.5% methylcellulose.

All mice survived to the end of the two-week observation period. There were no treatment-related clinical observations. All mice except one female gained body weight over the duration of the study. There were no gross pathologic lesions for any animal on study.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day feeding study with broiler chickens (Zeph, 2000)

The incorporation of maize was 54.21% for starter diets across all treatments and 57.03% for grower diets across all treatments.
There were no statistical differences in mortality, body weight, weight gain and feed conversion among the different treatments.

b) 90-day rat feeding study (MacKenzie, 2003).

Five groups of young adult male and female Crl:CD®(SD)IGS BR rats (12/sex/group) were administered diets containing 33% TC1507, 33% 33P66 (Near Isogenic Maize Grain), 33% 33J56 (Commercial Maize Grain), 11% TC1507, or 11% 33P66 (Near Isogenic Maize Grain) for approximately 90 days.
Under the conditions of this study, exposure of male and female rats to diets containing a transgenic strain of maize (TC1507) produced no toxicologically significant differences, compared to rats fed diets containing a non-transgenic, near isogenic strain of maize (33P66) or a non-transgenic commercial strain of maize (33J56). Male rats fed diet containing 33% TC1507 had slightly greater food consumption compared to rats fed diet containing 33% 33P66, but this was not considered toxicologically significant as it was not associated with significant differences in body weight gain or food efficiency.
In the document of MacKenzie (2003), only the Cry1F protein is mentionned. What about the PAT protein? Is the gene present in the plant and wasn't it mentionned by the author because the PAT protein is not detectable in grain, or was there an other reason?

c) 90-day rat feeding study (MacKenzie, 2007).

In the current study, 1507, near-isogenic control (33P66) and reference (33J56) maize grains were each used to produce separate batches of rodent feed according to the specifications of Purina Mills Certified Rodent LabDiet_ 5002.
These diets were fed to separate groups of rats for approximately 13 weeks. Over the duration of the feeding study, no biologically significant differences were observed in the in-life nutritional performance response variables between rats fed diets formulated with the 1507 maize grain and those fed the non-GM control diets. Additionally, there were no toxicologically significant differences in neurobehavioral, hematological, serum chemistry, urinalysis, organ weights, or pathology identified between rats consuming diets formulated with 1507 maize grain compared to rats consuming diets produced with non-GM maize grains.

d) Other relevant information (EFSA, 2004)

Twenty lactating dairy cows were used in a single cross-over design in which there was 2 x 28-day feeding periods. The aim was to compare the effect of using maize silage and maize kernels derived from transgenic 1507 maize on feed intake and milk production when compared with maize silage and maize kernels derived from a non-GM control variety.
Diets contained on average 43.0 % DM maize silage and 22.1 % concentrate of which 70.2 % was in the form of ground maize. Other feed ingredients included alfalfa hay, soybean meal, and cotton seeds. The diet composition was analysed for proximates, minerals (Ca, P, Mg, K), mycotoxins and silage fermentation products and found to be similar for both treatment groups.
CRY1F was detected in transgenic maize kernels and silage. PAT was not detectable in kernels, and ranged from not detectable to slightly above the detection threshold in forage, of 1507 maize.
The following measurements were made: (1) Physical (weekly): body weight, condition, temperature, pulse, feed intake; (2) Milk production (daily); (3) Milk composition (weekly): protein, fat, dry matter, lactose, urea N, somatic cell count, CRY1F; (4) Blood analysis (prior to and at the end of both trials): chemical and haematological. One cow was positive for the presence of CRY1F in milk prior to and during both treatments, which can therefore be considered a false positive ELISA-reaction. Results showed no significant differences between dietary treatments.

D.7.9 Allergenicity

NOT APPLICABLE

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

The study of Zeph (2000) with broilers does not provide information on the variability within treatments, so that the power of the statistical method cannot be calculated. Moreover, overall mortality rate and feed conversion are rather high, while growth rate is rather low.

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

Comments/Questions of the expert(s)

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

NOT APPLICABLE

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

NOT APPLICABLE

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

NOT APPLICABLE

D.11. ENVIRONMENTAL MONITORING PLAN

NOT APPLICABLE

References

none