Biosafety Advisory Council

Secretariat

O./ref.: WIV-ISP/BAC/2007_SC_536
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Title: Advice of the Belgian Biosafety Advisory Council on the application **EFSA/GMO/NL/2005/12** of Pioneer Hi-Bred/Mycogen Seeds under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2005/12 was submitted by Pioneer Hi-Bred/Mycogen Seeds in January 2005 for the marketing of food or feed products produced from or containing ingredients produced from of the insect resistant genetically modified maize 59122 under Regulation (EC) No. 1829/2003[1]. It has been officially acknowledged by EFSA on 16 September 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 absence of the necessary resources the Belgian Biosafety Advisory Council didn't take part in this consultation.

However, in early 2006, the Belgian Biosafety Advisory Council was in the position to contact experts to assist in the evaluation of the dossier in order to give advice to our minister. These experts were chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology. Four experts answered positively and assisted in the evaluation. The evaluation took place under the supervision of a coordinator who is a member of the Council.

The comments received from the Belgian experts (see Annex I for an overview of all the comments) are synthesised below by the coordinator.

[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)

The opinion of EFSA's scientific panel on GMOs was adopted on 23 March 2007 (The EFSA Journal, 2007, 470, 1-25)[2]

On 17 April 2007 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 on the EFSA opinion, and asked specifically, based upon their knowledge of the dossier, whether there are essential points in the dossier have not been taken into account in the opinion of EFSA.

Scientific evaluation

According to the Belgian experts, no major risks were identified neither concerning the molecular characterisation, the toxicity or the environmental risk[3].

The Biosafety Advisory Council (BAC) wants however to draw the attention of the Competent Authority to the following minor issues, which could be addressed to EFSA.

- This dossier concerns both food and feed applications. The notifier has done an analysis of the composition of the maize in line with the recommendations of the OECD consensus document on compositional considerations for new varieties of maize. That is, they have done a proximate analysis as recommended for human food purposes and they have analysed the fiber content by an ADF and NDF analysis as recommended for animal feed purposes. Some of our experts recommend a more detailed analysis of the carbohydrate fraction since this fraction constitutes the major part of the maize kernel. They also recommend an analysis of total, soluble and insoluble dietary fiber because this analysis is more relevant for food applications than an ADF and NDF analysis. Some experts would welcome information on the potential accumulation of transgene proteins in processed GM plant material.

- Because this dossier concerns only import and processing of maize for food and feed purposes, and maize kernels are very unlikely to establish themselves in areas where the imported maize will be handled or transported there are limited requirements for the general surveillance in this case. The BAC however would welcome a general surveillance plan which goes beyond a general frame and principles.

[2] see: <http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/809.html>

[3] As the application doesn't imply a cultivation of the plant in EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

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:

- Agrees with the GMO panel of EFSA that it is unlikely that maize 59122 will have any adverse effect on human and animal health or on the environment in the context of its intended uses.
- Would like to ask the Competent Authority to convey the above mentioned recommendations to the experts of EFSA.

p.o.

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

Annex I : Full comments of experts in charge of evaluating application EFSA/GMO/NL/2005/12 (ref: BAC_2007_PT_537)

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Bioveiligheidsraad
Conseil de Biosécurité

13 june 2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2006/PT_537[1]
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Expertise report for the EFSA dossier EFSA/GMO/NL/2005/12 - Compilation of all the comments received from the experts

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 12 december 2005
Coordinator: Prof. dr. ir. Dirk Reheul (UGent)
Experts: Yann Devos (UGent), Michel Paquot (FUSAGx), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: ecology, nature conservation, biodiversity, sustainable development, biosafety research, risk analysis, biochemistry of food/feed, industrial processing, novel food, toxicology, immunology, consumer info, genome analysis, genetic engineering, maize
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2005/12** concerns a notification of the company **Pioneer Hi-Bred/Mycogen Seeds** for the marketing of the genetically modified **maize MIR59122** for food and feed applications under Regulation (EC) 1829/2003.
The notification has been officially acknowledged by EFSA on 16 September 2005.
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 notification on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. Its was expected that the expert should evaluate if the information provided in the notification is sufficient in order to state that the marketing of the genetically modified plant for

[1] revised version of document BAC_2006_PT_358 completed with comments of the coordinator.

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.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The product described is 59122 maize for all food and feed uses, and for all food, feed and processed products derived from 59122 maize.
The 59122 maize has been genetically modified to express the Cry34Ab1, Cry35Ab1 and PAT proteins. The Cry34Ab1 and Cry35Ab1 proteins act together in the control of certain coleopteran insect pests, such as corn rootworm larvae (Diabrotica spp.). Expression of the PAT protein, used as a selectable marker, confers tolerance to the application of glufosinate-ammonium herbicide.
The product described in this application also consists of maize products from progeny, containing the genetic modification, as derived from conventional breeding between 59122 maize and traditionally bred maize.

Comment 2

The intended use of the product is similar to other conventional maizes. By consequence, food uses are concerned. Among these uses, the whole grains can be considered but also cracking of the grains in order to obtain mainly starch.
Native starch is a food ingredient but other important products are derivatives of starch : pregelified, modified or hydrolysed.
Following Syngenta, majority of maize is used for animal feeds, and about 8% of the grain is processed for human food products mainly by wet-milling or dry-milling. Nevertheless the food consumption of starch seems to be very high because starch (from maize, wheat,…) is a very important food ingredient. Maize grain is also processed into industrial products (11%), such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil. There are multiple categories of users of 59122 maize, e.g. animal feed and milling industry, agriculture, skilled trades and consumer use by public at large.

Comment 3

Sufficient information / No questions.

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

Comments/Questions of the expert(s)

Sufficient information / No questions.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Sufficient information / No 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)

Sufficient information / No questions.

D.2. Information on the sequences actually inserted or deleted

Comments/Questions of the expert(s)

Detailed information / No questions.

D.3. Information on the expression of the insert

Comments/Questions of the expert(s)

Comment 1

The expression level of the Cry34Ab1, Cry35Ab1 and PAT proteins in a range of tissues from 59122 maize representing key developmental stages of a typical maize plant (Iowa State University, 1993) was determined using specific Enzyme Linked Immunosorbent Assay (ELISA) systems developed for each protein.

At six locations in Chile (2002-2003), three locations in the USA (2003) and two locations in Canada (2003), samples of leaf tissue and root tissue were taken at the V9 (nine leaf stage), the R1 (silking stage, 50% pollen shed), the R4 (24-28 days after silking) and the R6 (physiological maturity) developmental stage. In addition, root samples were also taken at the V6 developmental stage in the USA and Canada locations. Samples of pollen and stalk were collected at approximately the R1 stage of development. Whole plants were collected at the V9 stage, the R1 stage and at the R6 stage. Mature grain (R6 stage) and forage (R4 stage) were also tested for level of Cry34Ab1, Cry35Ab1 and PAT protein expression.

For the Chile field trials, the mean expression level of the Cry34Ab1 protein in 59122 maize grain was 49.7 ng Cry34Ab1 protein/mg tissue dry weight. The mean expression level of the Cry35Ab1 protein in 59122 maize grain was 0.99 ng Cry35Ab1 protein/mg tissue dry weight. For all 59122 maize grain samples analysed, expression of the PAT protein was below the lower limit of quantisation of the PAT

ELISA used in this study. The lower limit of quantisation of the PAT ELISA for grain in this study was 0.06 ng/mg tissue dry weight.
The Cry34Ab1, Cry35Ab1 and PAT proteins were not detected in any tissue of the non-GM control maize.

For the USA and Canada field trials, the mean expression level of the Cry34Ab1 protein in sprayed 59122 maize grain was 39.6 ng Cry34Ab1 protein/mg tissue dry weight. The mean expression level of the Cry35Ab1 protein in sprayed 59122 maize grain was 1.98 ng Cry35Ab1 protein/mg tissue dry weight. The mean expression level of the PAT protein in sprayed 59122 maize grain was 0.1 ng PAT protein/mg tissue dry weight.
For the USA and Canada field trials, the mean expression level of the Cry34Ab1 protein in non-sprayed 59122 maize grain was 36.4 ng Cry34Ab1 protein/mg tissue dry weight. The mean expression level of the Cry35Ab1 protein in non-sprayed 59122 maize grain was 2.0 ng Cry35Ab1 protein/mg tissue dry weight. The mean expression level of the PAT protein in non-sprayed 59122 maize was 0.1 ng PAT protein/mg tissue dry weight.

The Cry34Ab1, Cry35Ab1 and PAT proteins were not detected in any tissue of the non-GM control maize.

Comment 2

The expression of Cry34Ab1 and Cry35Ab1 proteins are in grain. These proteins could be found in food derivative products. Even if the content is low, it is useful to check the risk of accumulation into specific derived products, especially those obtained by cracking of the raw materials. In other word, can the content in Cry34 Ab1, Cry 35 *Ab1 and* PAT increase in a product of cracking for the food chain. Where does these proteins partition (starch fraction, oil,…) ?

Comment 3

Sufficient information / no questions.

D.4. Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability

Comments/Questions of the expert(s)

Sufficient information / No questions.

D5. Genetic stability of the insert and phenotypic stability of the GM plant

Comments/Questions of the expert(s)

Sufficient information / No questions.

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

Comments/Questions of the expert(s)

Chances of genetic transfer to another organism are negligible.

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)

A comparison of the composition of 59122 maize grain and grain from a non-GM control maize with comparable genetic background has been carried out.
Composition data were obtained from field trials carried out at six locations in Chile during the 2002-2003 growing season, from field trials carried out at three locations in the USA during 2003 and from field trials carried out at two locations in Canada during 2003.
Each location included a randomised block design containing four blocks. In the Chile field trials, each block contained the 59122 maize and a non-GM control maize with comparable genetic background for comparative purposes. In the USA and Canada field trials, each block contained non-sprayed 59122 maize, sprayed 59122 maize (sprayed with glufosinate-ammonium herbicide) and a non-GM control maize with comparable genetic background for comparative purposes. Grain samples for compositional analysis were collected from three out of the four blocks per location. The remaining block was used for expression analysis of the Cry34Ab1, Cry35Ab1 and PAT proteins in 59122 maize tissues.
Grain samples for compositional analysis were taken as follows. Grain samples were collected once the plant had reached physiological maturity (R6 stage) from blocks 2, 3 and 4 at each location. Plants used for grain collection were self-pollinated.
Five individual ears, comprising one sample, were collected from the 59122 maize and the non-GM control maize in the Chile field trials. In the USA and Canada field trials, five individual ears, comprising one sample, were collected from the non-sprayed 59122 maize, the sprayed 59122 maize and the non-GM control maize.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Annex 3. presents an important study on the nutrient analysis of 59122 maize grains and an non-GM control maize grain.

In general, no statistically significant differences were observed across locations between the GM and the non-GM maize grain. Statistically significant differences were observed for some components (crude proteins, ash, carbohydrates, calcium, some fatty acids,…) in the analysis across locations in Chile. However, generally a significant difference was only observed at one of the six individual locations at the maximum. No significant differences were observed for secondary metabolites in Chile.
On the contrary, statistically significant differences for mean secondary metabolites and anti-nutrient composition were observed from the USA and Canada locations across location but were not consistently observed when evaluated per individual locations.
In general no statistically significant differences across locations were observed for other constituents except for mean vitamin and threonine.
Regarding the analytical method, some comments are to be done :

- the main compound, the carbohydrates, are only obtained by difference and the analysis of starch is missing while it is the main carbohydrate component.
- Considering food application, the analysis of dietary fibre by acid detergent *fibre (ADF)* and neutral dietary fibre (NDF) is not recommended. The evaluation of total dietary fibre, soluble and insoluble one would be more obvious.

Dietary fibres may be composed of soluble and insoluble constituents. Because of the large diversity indigestible materials, analysis is difficult. Enzymatical methods are preferable to the Van Soest method even if this last technique has been standardized in some countries, especially for cereals.
The Van Soest method gives values similar to those obtained *in vivo* from digestibility studies with animals. This technique allows to determine the concentration of cellulose, lignin and hemicelluloses. Nevertheless, the Van Soest method does not correspond to the actual notion of dietary fibres including a lot of other constituents as soluble and insoluble fibres are not distinguished.
With enzymatical methods the digestible constituents (1-4 β-glucans, proteins) in the defatted sample are enzymatically hydrolysed (heat stable α-amylase, gluco-amylase, protease). Water soluble fibres are isolated by precipitation with ethanol. The proteins and mineral matter still remaining with the soluble and insoluble dietary fibres are deducted.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

D.7.5 Product specification

Comments/Questions of the expert(s)

Following the requesting, all human food and animal feed from 59122 maize can be considered to be substantially equivalent to all human food and animal feed derived from commercial maize with no nutritionally or toxicologically significant charges.

Even if differences between the two maize are not numerous and quantitatively important, a rigorous equivalence cannot be guarantied from the analysis. Toxicological studies seem necessary.
Comparison with reported literature ranges is not valid because of the too high reported variability. Analytical methods do not comprise starch and dietary fibre. The presence of newly expressed proteins must be analysed (toxicology, partition during food process see 7.8.1). As the transgenic plant inactivates the herbicide, metabolised products might be present in the plant. Are there degradation products from the herbicides in maize ?

D.7.6 Effect of processing

Comments/Questions of the expert(s)

The 59122 maize will undergo existing methods of production and manufacturing used for commercial maize. No novel method of production and manufacturing is envisaged. In the EU, most of the maize is used for animal feed, and only about 8% is processed into food products such as highly refined starch by the wet-milling process and maize flour by the dry-milling process. The majority of the starch is used for sweeteners and fermentation including high fructose maize syrup and ethanol. In addition to milling, the maize germ can be processed to obtain maize oil. These processed products of maize are used in a variety of food.

The cracking of maize in order to produce different food ingredients especially starch, oils and proteins, may concentrate some secondary metabolites or anti nutrients and allergens (9 kd lipid transfer protein, 16 kd trypsin inhibitor,….).
Some of these compounds will be denatured during food processes especially by heat treatment. It is the same for the proteins CRY34 AB1, CRY35AB1 and PAT.
Nevertheless, the chemical composition of a raw material is not sufficient to predict the repartition of the minor components during a food process or the cracking.
The used technology is of course important but also the locations and the interactions between the different components in the raw materials.
Considering the difficulties to conduct such studies and, on the other hand, the existence of a risk, even weak, to concentrate a secondary metabolite in an other way than the traditional sources, recommendations of a post-market monitoring seem necessary.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

The estimated consumption of maize by the European population is 8,8 g/person/day in the dossier. It is not specified if this estimation takes into account a lot of food products containing ingredients from cracking (starch especially, oils, ….). The consumption at the 95th percentile is not also specified. These estimations are necessary for the food application.

The key nutrients have been analysed except the carbohydrates fraction (simple and complex), starch and dietary fibre.

See also D.7.3.

The carbohydrates fraction is obtained by difference and not characterized excepted for raffinose. Dietary fibre is estimated via ADF and NDF, not relevant for food use.

Even if the genetic modification does not change significantly the overall nutrient, investigations to what extent the different industrial processes can lead to the concentration or the elimination of the minor constituents are useful. Otherwise a surveillance programme should accompany the marketing of derived products from 59122 maize.

Additionnal comment from the coordinator
The dossier specifies in which circumstances the 8,8 g/person/day are considered.

D.7.8 Toxicology

Comments/Questions of the expert(s)

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Screening for structure-activity relationship, *in vitro* digestibility assays, and acute toxicity testing.
The safety in terms of toxicity for the PAT protein has already been determined in detail during the assessment of glufosinate-ammonium tolerant maize (Annex 1). Agreed.
A battery of tests showed that the newly expressed proteins Cry34Ab1, Cry35Ab1, and PAT are structurally and functionally not related to known toxins (Annex 16) and food allergens (Cry34Ab1, Cry35Ab1 only: Annex 19). The Cry34Ab1 and Cry35Ab1 proteins were found sensitive to heat (Annex 18) and are rapidly degraded under simulated mammalian gastric conditions (Annex 17). Agreed.

The potential toxicity of the 59122 maize expressed Cry34Ab1 and Cry35Ab1 proteins to humans and animals was examined in acute oral toxicity studies in mice either separately or as a Cry34Ab1/Cry35Ab1 protein mixture (microbe-derived equivalent proteins were used). In the acute oral toxicity studies of the Cry34Ab1 protein (2700 mg/kg bw), the Cry35Ab1 protein (1850 mg/kg bw), and Cry34Ab1/Cry35Ab1 protein mixture (482 mg/kg bw; 1520 mg/kg bw), no mortality occurred. No adverse clinical signs were observed during the study and no treatment-related gross pathologic findings noted at necropsy (Annex 11, 12, 13). Although weight loss was observed between test days 1 and 2 (Cry34Ab1 study: 3 of 5 animals; Cry35Ab1 study: 2 of 5 animals; Cry34Ab1/ Cry345Ab1 study: 2 of 10 animals), probably due to gavage with a maximum volume of methylcellulose, it was concluded that there were no test-substance related effects on bodyweight. Microscopic pathology was not performed. In conclusion, the proteins were found not acutely toxic in mice. Agreed.
The Cry34Ab1 and Cry35Ab1 proteins used for the *in vitro* digestibility studies and the acute oral toxicity studies were produced by *Pseudomonas fluorescens*. The PAT protein to perform regulatory studies was produced by *Escherichia coli*. The microbial test substances were compared by various structural, functional and biochemical parameters to the Cry34Ab1, Cry35Ab1, and PAT proteins produced in the transgenic maize event 59122 (Annex 9, 10). The microbe-derived recombinant Cry34Ab1, Cry35Ab1 and PAT proteins and the 59122 maize-derived proteins are found substantially equivalent and the microbial test substances can be accepted as a suitable surrogate. Agreed.
Repeated dose toxicity testing.
No 28-day oral toxicity test has been performed with either Cry34Ab1 or Cry35Ab1 proteins. This was not motivated by the applicant. As no safety concerns have been raised by the acute toxicity studies, digestibility and thermolability studies, toxin homology searches, or the whole-food toxicity testing performed in the 90-days feeding study in rats and the 42-days broiler chicken study, the proteins can be considered non-toxic and unlikely to present a health risk to humans and animals. We decide not to request for a 28-days oral toxicity study combining the 2 proteins.
Genotoxicity testing.
Genotoxicity testing, whether it is performed or not, should always be well motivated.

Comment 2

The degradation of the protein by gastric fluid can be different in a complex food system.
The loss of toxicity against southern corn rootworm after exposure to heat treatment show only the denaturation of the protein in relation with this biological activity. For example, these experience do not prove a loss of potential allergenicity after heat treatment.

Study of potential allergenicity is necessary (D.7.9).
The thermolability of PS149B1 binary Delta-Endotoxin (appendix 18) was conducted at 60, 75 and 90° for 30 minutes.

- the thermodenaturation could be different in a complex system and results could be different for pasteurised products (ex. 75°C, shorter time)
  protein are not denatured in the same way when comparing high temperature short time treatments against lower temperature long time. Denaturing and cooking effects are more important for long treatments. It is the same for sterilization (eg. 120°C - 20 min) against UHT (140°C- few seconds).

Additionnal comment from the coordinator
According to another expert questionned on this point, heating at high temperature for a short period has on the proteins the same denaturating effect than heating at 60, 75 and 90° for 30 minutes.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

No constituents other than the Cry34Ab1, Cry35Ab1, and PAT protein are novel. The absence of any harmful fusion protein is demonstrated by bioinformatics analysis (Annex 20). Agreed.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

The presence and levels of natural food and feed constituents have been analysed in grain from 59122 maize grown in Chile, USA, and Canada (sprayed/non-sprayed with glufosinate-ammonium herbicide) and compared with non-genetically modified control maize with comparable genetic background and data from literature (Annex 3, 4). Although sporadic statistically significant differences were observed between 59122 maize grain and isogenic controls, these changes were not found biologically significant (no consistent patterns). In conclusion, compositional analysis including key macro- and micro-nutrients, toxicants, anti-nutrients and other constituents of grain from 59122 maize grown at different geographical regions and from more than one growing season show that 59122 maize grain is comparable to grain from non-genetically modified control maize with comparable genetic background. No particular natural constituents of 59122 maize are considered to be of significant concern to require additional information or further risk assessment. Agreed.

Comment 2

See D.7.5 and D.7.6

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

The 90-days whole food study in rats. (Annex 14)
A 90-days oral toxicity feeding study in rats has been carried out with 59122 maize grain in order to confirm the absence of toxicity of the Cry34Ab1, Cry35Ab1, and PAT proteins expressed in 59122 maize grain. In this well-conducted sub-chronic toxicity study, no consistent differences in bodyweight, food consumption, clinical condition, opthalmological observations, neurobehaviour, clinical pathology, organ weights or histopathology were noted for rats fed on 35% 59122 maize and rats fed on 35% non-GM control maize with comparable genetic background, or a commercial non-

GM maize. It was concluded that 59122 maize grain did not cause any diet related effects and there were no differences with the non-GM maize. Agreed.
The 42-days poultry feeding study. (Annex 15)
The 42-days poultry feeding study was conducted to evaluate the nutritional value of 59122 maize grain compared with grain from non-GM control maize with comparable genetic background, and grain from 3 commercial non-GM maize. The results show that there were no biologically relevant differences in the parameters tested (mortality, body weight and body weight gain, feed efficiency, overall organ yield, carcass yield, breast, thigh, wing, and leg yield, abdominal fat) between broilers fed the 59122 maize grain and the non-GM control maize diet. Agreed.
In conclusion, no differences in wholesomeness are expected with comparable non-GM maize varieties.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

The estimated consumption of maize by the European population is 8,8 g/person/day in the dossier. It is not precised if this estimation takes into account a lot of food products containing ingredients from cracking (starch especially, oils, ….). The *consumption* at the 95th percentile is not also precised. These estimations are necessary for the food application.
The key nutrients have been analysed except the carbohydrates fraction (simple and complex), starch and dietary fibre.

See also D.7.3.

The carbohydrates fraction is obtained by difference and not characterized excepted for raffinose. Dietary fibre is estimated via ADF and NDF, not relevant for food use.

Potential toxicants and anti-nutrients have been measured but no information is given about the effect of processing (derived food products) upon the content and distribution of nutrients and anti-nutrients considering that a substantial equivalency with traditional maize is argued.

Even if the genetic modification does not change significantly the overall nutrient, investigations to what extent the different industrial processes can lead to the concentration or the elimination of the minor constituents are useful. Otherwise a surveillance programme should accompany the marketing of derived products from 59122 maize.

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

Comments/Questions of the expert(s)

No risks to human health have been identified by comparison of commercial maize. The substantial equivalency of 59122 maize and commercial maize is argued.
It should be recommended to have a post-market monitoring taking into account previous comments.

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

Comments/Questions of the expert(s)

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)
See 9.3

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)
See 9.3

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

- Establishment of invasive feral DAS-59122-7 maize populations (vertical gene flow): unlikely.
During transport of the imported maize F_2 grains some grains will inevitably be spilled accidentally. The probability that these spilled grains result in self-sustaining and/or invasive feral maize populations is *nihil*. Maize has been grown for many years in the EU and feral maize populations are rarely observed. Even in agricultural fields with ideal conditions, maize is not known to persist as a weed: volunteers are killed by frost. In Mediterranean countries, some volunteers occur but these are easily controlled by current agronomic practices. Due to its high level of domestication, the maize germination and establishment potential outside the fields is expected to remain very low despite the acquisition of the new transgenic traits (that confer a selective advantage under field conditions with the appropriate selection pressure). It is important to bear in mind that the imported grains that can be spilled are from the F_2 generations and that these are expected to be less fit than F_1 grains. Moreover,

the selection pressure of corn rootworm is currently small in Belgium being limited to some major airports but it may increase in the future.

- Acquisition of transgenic traits by wild/weedy maize relatives resulting in invasive progeny (vertical gene flow): unlikely.
There are no wild/weedy relatives of maize in the EU. However, cultivated maize occurring in the neighbourhood of the feral DAS-59122-7 maize could theoretically act as a recipient. This hypothesis seems not very probable. First, feral maize populations resulting from spilled grains are rarely observed and it is even more doubtful that these plants will flower. Second, pollen flow would only occur locally. The pollen amount will be small compared to a field of commercial size. As maize pollen is heavy, pollen will be deposited within a few meters of the source. Third, it is not clear that feral maize plants will flower and release their pollen at the moment of silking of the recipients.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

- Corn rootworm develops resistance requiring alternative pest control: unlikely.
- Other pests take become abundant due to the efficient control of corn rootworm: unlikely.
The number of spilled DAS-59122-7 grains and feral DAS-59122-7 plants will be extremely low and the selective pressure against the corn rootworm small.

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

Comments/Questions of the expert(s)

- Decline of non-target organisms resulting in the loss of biodiversity and/or ecological functions: unlikely.
The number of feral DAS-59122-7 plants will be small as a result of what the environmental exposure of DAS-59122-7 will be extremely low.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

- Accidental consumption of maize plant parts leading to toxicological and/or allergenic reactions: unlikely.
See food and feed experts.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

- Accidental consumption of maize plant parts leading to toxicological and/or allergenic reactions: unlikely.
See food and feed experts.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

- Decline of non-target organisms resulting in the loss of biodiversity and/or ecological functions: unlikely.
- Impact on carbon and nitrogen recycling through changes in soil decomposition of organic material: unlikely.
The exposure to spilled DAS-59122-7 grains and feral DAS-59122-7 plants will be extremely low as a result of what the environmental exposure to toxins produced by DAS-59122-7 (*e.g.* through root exudates and decaying plant parts) will be extremely low.

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

Comments/Questions of the expert(s)

- Not of application.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

The general surveillance (GS) remains very vague: a general frame of principles is not even provided. It might be advisable to complete the GS with more details once a positive advice of EFSA is obtained (*e.g.* include the names and coordinates of the networks that will be involved in the GS, provide the countries/regions where the GS will be done).

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

There is no need to foresee a case-specific monitoring (CSM), since the risks were extremely low and no uncertainties remained in the ERA. CSM is not mandatory but required when uncertainty remains.

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

Comments/Questions of the expert(s)

On the opposite of the CSM, GS is mandatory for viable GM material. Non-viable GM material falls beyond the scope of the environmental monitoring provision. In case of imported viable grains, GS should consider that if substantial loss, spillage and establishment are possible, appropriate management systems should be in place to restrict environmental exposure. Given the increasing number of notifications covering import, unforeseen (cumulative) effects should be followed up in the GS.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

References

Eastham K, Sweet J (2002) Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Environmental Issue Report 28, EEA, http://reports.eea.eu.int/environmental_issue_report_2002_28/en/GMOs%20for%20www.pdf