COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de staatssecretaris van
Infrastructuur en Milieu
Dhr J.J. Atsma
Postbus 30945
2500 GX Den Haag

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KENMERK CGM/120104-02
ONDERWERP Import van genetisch gemodificeerde sojalijn FG72 met glyfosaat en isoxaflutole herbicidentolerantie

Geachte heer Atsma,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/BE/2011/98 voor de import en verwerking van genetisch gemodificeerde sojalijn FG72, ingediend door Bayer CropScience AG, adviseert de COGEM als volgt.

Samenvatting

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde sojalijn FG72. In deze lijn komen de genen *2mepsps* en *hppdPfW336* tot expressie wat resulteert in een sojaplant tolerant voor glyfosaat en isoxaflutol bevattende herbiciden.

Het Nederlandse klimaat is niet optimaal voor sojateelt. Tijdens de zomer zijn de dagen lang, terwijl soja een korte dagplant is die korte dagen nodig heeft voor bloei en ontwikkeling. Om die reden wordt soja op dit moment niet geteeld in Nederland. Er zijn echter initiatieven voor de ontwikkeling van extreem vroeg bloeiende sojarassen die kunnen groeien in het gematigde Nederlandse klimaat. Gezien de eigenschappen van soja, is de COGEM van mening dat deze ontwikkeling geen aanleiding geeft tot extra risico's. Soja beschikt niet over eigenschappen die nodig zijn voor verwildering zoals zaadverspreiding, kiemrust en kouderesistentie. Daarnaast worden opslagplanten wereldwijd zelden in teeltgebieden waargenomen. Er zijn geen redenen om aan te nemen dat de geïntroduceerde eigenschappen in FG72 het verwilderingspotentieel vergroten. In Europa zijn er geen wilde verwanten van soja aanwezig, waardoor uitkruising niet mogelijk is.

Op grond van de genoemde argumenten acht de COGEM de kans dat incidenteel morsen in Nederland tot verspreiding van FG72 leidt, verwaarloosbaar klein. De COGEM is van mening dat de moleculaire karakterisering adequaat is uitgevoerd. Hoewel de COGEM het door de aanvrager opgestelde 'general surveillance' plan voor monitoring onderschrijft, ziet zij een enkel punt voor verbetering.

Op basis van de genoemde overwegingen acht de COGEM de risico's van import en verwerking van sojalijn FG72 verwaarloosbaar klein. Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ir. Bastiaan C.J. Zoeteman
Voorzitter COGEM

c.c. Dr. I. van der Leij
Drs. H.P. de Wijs

Import of genetically modified soybean FG72 with glyphosate and isoxaflutole herbicide tolerance

COGEM advice CGM/120104-02

Summary

The present application of Bayer CropScience AG (EFSA/GMO/BE/2011/98) concerns the import and processing for use in feed and food of genetically modified soybean FG72. Cultivation is not part of this application.

Soybean line FG72 was obtained by direct gene transfer of the transgenic insert to conventional soybean cells. The line expresses the 2mepsps *gene and the* hppdPfW336 *gene conferring tolerance to several herbicides.*

In Europe, there are no wild relatives of soybean and therefore, hybridisation with other species is not possible. Soybean does not possess any of the attributes commonly associated with problematic weeds such as seed shattering, dormancy and cold resistance. Establishment of feral soybean populations has never been observed in Europe. In addition, soybean volunteers are rarely observed throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers in managed ecosystems. COGEM is of the opinion that the environmental risk of spread of soybean FG72 within the Netherlands due to incidental spillage of this herbicide tolerant soybean line is negligible.

In the opinion of COGEM, the molecular analysis of soybean line FG72 is adequately performed. Although the general surveillance (GS) plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current GS plan sufficient for import and processing of soybean line FG72.

In conclusion, COGEM is of the opinion that import and processing of soybean line FG72 poses a negligible risk to the environment. COGEM points out that a food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present notification EFSA/GMO/BE/2011/98 by Bayer CropScience AG concerns import and processing of the genetically modified soybean line FG72. This soybean line was produced by direct gene transfer of the transgenic insert to conventional soybean cells. FG72 soybean expresses the *hppdPfW336* gene from *Pseudomonas fluorescens* and the *2mepsps* gene derived from maize. These genes confer tolerance to the herbicide isoxaflutole (IFT) and glyphosate-containing herbicides, respectively.

Previous COGEM advice

COGEM has not advised on plants with IFT herbicide tolerance traits before.

The *2mepsps* gene has been assessed by COGEM before, as it is present in GA21 maize and GHB614 cotton. COGEM has advised several times on maize line GA21, regarding both import and cultivation of the line itself, and import of hybrid maize lines including GA21.[1,2,3,4,5,6] In 2008 and 2011, COGEM advised on the import and processing of cotton line GHB614 and the hybrid line GHB614xLLcotton25, respectively.[7,8] In the referred cases, COGEM concluded that there were no indications that the lines posed any risks to the

environment. Since 2008 maize GA21 and since 2011 cotton GHB614 are authorised for import and processing in the European Union.[9,10]

Aspects of the crop

Soybean (*Glycine max*) is a member of the genus *Glycine* and belongs to the *Fabaceae* (*Leguminosae*) family. Soybean is grown from equatorial to temperate zones. The optimum temperature for soybean growth is between 25°C and 30°C. Soybean seeds will germinate when the soil temperature reaches 10°C and under favourable conditions a seedling will emerge in a 5-7 day period. Soybean is sensitive to frost and therefore does not survive freezing conditions.[11,12]

In the Netherlands, frost is common. On average 58 days in a year have a minimum temperature below 0°C.[13,14] In the summer days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering. The Dutch climate is therefore not optimal for cultivation of soybean. However, field trials with a number of soybean varieties have shown that cultivation of soybean is possible.[15,16] Further improvement of these varieties may result in soybean varieties suited for commercial cultivation in the Netherlands. Due to the characteristics of soybean, COGEM is of the opinion that this development does not affect the environmental risk assessment.

The soybean plant is not weedy in character.[11] As for all domesticated crops, soybean has been selected against seed shattering to reduce yield losses during harvesting. Soybean seeds rarely display dormancy and poorly survive in soil.[17] Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers in managed ecosystems.[11] In addition, volunteers are easily controlled mechanically or chemically.[11] COGEM is not aware of any reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.[11] The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower. In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[11]

Molecular characterization

FG72 was developed by direct gene transfer of a transgenic insert into cells of conventional soybean line 'Jack'. The transgenic insert is a purified *Sal*I restriction fragment from plasmid pSF10. This insert contains two expression cassettes, conferring tolerance to glyphosate-containing herbicides and the herbicide IFT. The expression cassettes are located adjacent to each other in opposite orientation. An overview of the transgenic insert introduced in FG72 is given below:

- 3'nos untranslated region including a transcription terminator, derived from the nopaline synthase gene from *Agrobacterium tumefaciens*;
- *hppdPfW336* gene, encoding a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD) of *P. fluorescens*;
- TPotp Y optimized chloroplast transit peptide, based on sequences from sunflower (*Helianthus annuus*) and maize (*Zea mays*);
- 5'TEV leader sequence originating from *Tobacco etch virus*;
- Ph4a748 ABBC sequence including the promoter region of the histone H4 gene of *Arabidopsis thaliana*. The sequence contains an internal duplication;

- Ph4a748 sequence including the promoter region of the histone H4 gene of *A. thaliana*;
- Intron1 h3At, first intron of gene II of the histone H3.III variant of *A. thaliana*;
- TPotp C optimized chloroplast transit peptide based on sequences from sunflower (*H. annuus*) and maize (*Z. mays*);
- *2mepsps*, modified *epsps* gene originally derived from *Z. mays*, encoding a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS);
- 3'histonAT, untranslated region including a transcription terminator, derived from the histone H4 gene of *A. thaliana*;

Expressed proteins

FG72 soybean expresses a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD) originating from the ubiquitous bacterium *P. fluorescens*. HPPD is an enzyme native to all eukaryotic organisms. It is involved in the catabolism of the amino acid tyrosine to components important in photosynthesis and vitamin E production. Although HPPD from *P. fluorescens* has only a 21% amino acid sequence identity with plant HPPD, conserved sequences are maintained between the proteins.[18] To reduce the sensitivity of soybean to HPPD inhibitors, such as the herbicide IFT, the amino acid tryptophan at position 336 of the *P. fluorescens* HPPD protein is substituted for a glycine. A chloroplast transit peptide (TPotp Y) is fused to the *hppdPfW336* gene to ensure the transport of the expressed protein to the chloroplast.

FG72 soybean also contains the *2mepsps* gene. The *2mepsps* gene was generated by introducing mutations into the wild-type *epsps* gene from maize, leading to a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) with two amino acid substitutions (2mEPSPS). A chloroplast transit peptide sequence (TPotp C) is fused to the *2mepsps* gene, resulting in the transport of the 2mEPSPS protein to the chloroplast.[19]

EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids and is active in the chloroplasts of a plant cell. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants.[20,21] The 2mEPSPS enzyme has a decreased binding affinity of the protein for glyphosate. In addition, a methionine amino acid is added at the N-terminal site of the protein to restore the cleavage site of the chloroplast transit peptide.

Molecular analysis

The applicant demonstrated by Southern blot analyses that two copies of the transgenic insert are integrated in the genome of FG72. Upon integration of the insert into the soybean genome, a genomic region translocated to a new position. Additionally, Southern blot and PCR analyses showed that the backbone sequences of plasmid pSF10 are absent in FG72.

Sequence analysis of the FG72 insertion site demonstrated that the flanking sequences adjacent to the insert, consist of genomic soybean DNA. At the 5' junction, two partial 3'histonAt sequences were integrated in a head to head orientation, followed by two complete transfer DNA copies arranged in a head to tail orientation. Upon integration of the insert into the genome, a non-transgenic genomic soybean region translocated to a new position at the 3' junction, which at its 3' end was joined by 158 bases of the Ph4a748 promoter sequence. After translocation, 25 bases of the translocated sequence and 2 bases of the reintegration site

were deleted. Furthermore, sequence analysis revealed a 24 bases insertion of unknown origin at the 3' junction of insert and genomic DNA.

DNA sequences spanning the junctions between the transgenic insert, the translocated sequence, and the plant genome were analysed for the presence of potential encoded fusion proteins. Sequences were analysed from stop to stop codon. Open reading frames identified were compared with sequences of known allergens deposited in the AllergenOnline database (February 2011) and to protein sequences deposited in the Bayer toxin database (June 2011). There were no indications for any sequence similarities between known toxins or allergens harmful to humans or animals.

In view of the above, COGEM is of the opinion that the molecular characterisation of FG72 has been adequately performed and meets the criteria laid down by COGEM.[22]

Environmental risk assessment

The current application concerns import and processing of soybean line FG72. In case of spillage soybean seed may be released into the environment. Soybean seeds rarely display dormancy, poorly survive in soil and do not survive freezing winter conditions. The Dutch climatic conditions are not optimal for growth of soybean. In the summer days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers.[11] In addition, volunteers are easily controlled mechanically or chemically.[11] Field trials with FG72 soybean focusing on agronomic and phenotypic characteristics did not give any indication of increased weediness due to the presence of the HPPD W336 and 2mEPSPS proteins.

In view of the above, COGEM is of the opinion that the environmental risk of spread of soybean FG72 within the Netherlands due to incidental spillage is negligible.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.[2] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General surveillance plan

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

The GS plan in this application states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorisation holder and operators involved in the handling and use of viable FG72 soybean. In 2010, COGEM formulated criteria for GS plans concerning applications for import and cultivation of GM crops.[23] Although the GS plan

could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the GS plan sufficient for import and processing of FG72 soybean.

Advice

COGEM has been asked to advise on import and processing for use in food and feed of soybean line FG72. This genetically modified soybean line expresses the *hppdPfW336* gene from *P. fluorescens* and the *2mepsps* gene derived from maize. These genes confer tolerance to the herbicide isoxaflutole (IFT) and to glyphosate-containing herbicides, respectively. The molecular characterization of soybean line FG72 meets the criteria of COGEM.

Although field trials have indicated that some soybean varieties can be cultivated in the Netherlands, the Dutch climate is not optimal for soybean growth.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers. Modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds such as seed shattering, dormancy, and cold resistance. There is no reason to assume that expression of the introduced *hppdPfW336* and *2mepsps* genes will increase the potential of soybean to establish feral populations. In addition, establishment of feral soybean populations in Europe has never been observed.

COGEM is of the opinion that the risk of spread of soybean FG72 within the Netherlands due to incidental spillage of this soybean is negligible. Wild relatives of soybean are not present in Europe and therefore introgression of the inserted gene into closely related species can not occur.

Although the GS plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current GS plan sufficient for import and processing of soybean line FG72.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean FG72 poses a negligible risk to the environment. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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