COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

Aan de Staatssecretaris van
Volkshuisvesting, Ruimtelijke
Ordening en Milieubeheer
De heer drs. P.L.B.A. van Geel
Postbus 30945
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datum	10 april 2006
kenmerk	CGM/060410-04
onderwerp	Advies Marktdossier EFSA/GMO/NL/2005/18 Herbicidetolerante soja A2704-12

Geachte heer van Geel,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/NL/2005/18, getiteld 'Glufosinate ammonium-tolerant soybean, A2704-12' voor de import en verwerking van genetisch gemodificeerde soja door Bayer CropScience GmbH adviseert de COGEM als volgt.

Samenvatting:
De COGEM is gevraagd te adviseren over import van genetisch gemodificeerde sojabonen en daarvan afgeleid voedsel en veevoer. De sojalijn is voorzien van het *pat* gen waardoor de planten tolerant zijn geworden voor bespuitingen met herbiciden met als werkzame stof glufosinaat-ammonium.
In Europa komen geen wilde verwanten van soja voor en soja bezit niet de eigenschappen om te kunnen verwilderen. Soja is sterk koudegevoelig en heeft hoge temperaturen nodig voor kieming en ontwikkeling van de plant. Er zijn geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot. De COGEM acht de kans verwaarloosbaar klein dat incidenteel morsen van de soja leidt tot verspreiding van deze genetisch gemodificeerde soja binnen Europa.
De COGEM merkt op dat de moleculaire karakterisering van de sojalijn onvolledig is. In het dossier ontbreken gegevens die duidelijkheid verschaffen over de 5' flankerende sequentie. Hierdoor kan niet volledig uitgesloten worden dat er ten gevolge van de insertie nieuwe open leesramen zijn ontstaan die eventueel tot toxische of allergene producten kunnen leiden. Gezien de onvolledige moleculaire karakterisering adviseert de COGEM negatief over deze aanvraag voor markttoelating.

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. ir. B.P. Loos
Dr. R. C. Zwart

Import and processing of herbicide tolerant soybean A2704-12

COGEM advice: CGM/060410-04

The present application by Bayer CropScience GmbH of file EFSA/GMO/NL/2005/18, 'Glufosinate ammonium-tolerant Soybean Transformation Event A2704-12' concerning the commercial import and processing for use in feed and food of a genetically modified soybean. Cultivation is not part of this application.

The recombinant soybean line is genetically modified by insertion of the pat gene. As a result, A2704-12 soybean confers tolerance to herbicides containing the active ingredient glufosinate ammonium. The soybean line is already commercially grown in the United States of America and Canada.

In Europe, no wild relatives of soybean are present and modern soybean cultivars do not possess any of the attributes commonly associated with problematic weeds. There is no reason to assume that the inserted genes will increase the potential of the soybean to run wild. Furthermore, establishment of feral populations in soybean producing countries is never observed. Soybean can not survive the climatological conditions in North-West Europe. COGEM is of the opinion that, soybean volunteers can not survive and establish themselves in the wild. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will pose no risk to the environment in the Netherlands nor in Europe.

COGEM points out that there is a lack of information regarding the molecular analysis. In particular incomplete data are presented on the 5' flanking sequence. On basis of the available data it can not be ruled out that new chimeric open reading frames were created due to the insertion. These putative open reading frames could give rise to potential toxic or allergenic products. In view of the deficiencies in the molecular analysis, COGEM advises negatively on this application.

Introduction

The present application by Bayer CropScience GmbH, file EFSA/GMO/NL/2005/18 'Glufosinate ammonium-tolerant Soybean, A2704-12' concerns the commercial import and processing for use in feed and food of genetically modified soybean (*Glycine max*).

The soybean event A2704-12 is genetically modified by the introduction and expression of the *pat* gene which confers tolerance to herbicides containing the active ingredient glufosinate ammonium.

A2704-12 soybean has been approved for commercial import, processing and cultivation in the United States of America and Canada in 1998 and 2000 respectively (1). Moreover, A2704-

12 has been approved for food use in Australia and New Zealand, for feed use in Russia and for food and feed use in Japan, Mexico and South Africa. There is a history of safe use e.g. no adverse health effects concerning handling and consuming of products and derivatives of this line have been reported. The genetically modified soybean line A2704-12 is cultivated in the United States of America and Canada. The harvested soybeans will be used for processing into animal feed and oil and protein isolates for human consumption. Whole soybean, oil, cake and meal derived from soybean event A2704-12 will be imported in the European Union and further processed.

Previous COGEM advices

In the past COGEM has advised positively on a genetically modified soybean line containing a *CP4 epsps* gene (glyphosate tolerance) for import (CGM/950629-16). The COGEM has advised positively on the use of the *pat* gene for import of 1507 maize (CGM/030115-01) and for the cultivation of Bt11 maize (CGM/030822-01).

Aspects of the crop

Soybean is a member of the genus *Glycine* and belongs to the *Fabaceae* (*Leguminosae*) family (2). Soybeans are adapted to agricultural regions from equatorial to temperate zones. Legumes, like soybean, are characterised by fixing atmospheric nitrogen as a source of nitrogen for growth and development in a symbiotic relationship with *Bradyirhizobium japonicum*. In new soybean production areas, inoculation of the seeds with this bacterium is necessary for an efficient root nodulating system (3).

Depending on cultivar and climate, the growth period can range from 65 to 150 days. The crop starts flowering 25 to 150 days after sowing, depending on the day length, temperature and cultivar. Flowering can take 1-15 days; pod formation takes 7-15 days; seed filling takes 11-20 days and ripening to harvest 7-15 days (2). Soybean is a short-day plant, which means that development to maturity usually is shorter with short days than with long days (2). The optimum temperature for the growth of soybean is between 25°C and 30°C. Soybean is very susceptible to frost damage and somewhat susceptible to excessive drought and extended flooding.

Cultivated soybean is an annual self-pollinating species. Seeds are the only structure of survival in soybean (4). The absence of dormancy in seeds causes a limited survival rate of seeds (3). Besides that, seeds are susceptible for climatic conditions (3). Due to a relatively large seed size, the dispersal of seeds is limited. Animal transportation is not encouraged by the morphological characteristics of the seedpod or seeds (4). Dispersal of seeds may occur by humans during transport, sowing or harvest (4). As soybean is a self-pollinating species, the dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (4). The estimated outcrossing rates for soybean vary between 0.5 and 1 percent (5). Insect pollinators cause natural cross-pollination between soybeans (3). Soybean

volunteers are rare and the cultivated soybean plant has never been found outside of cultivation (3). There are no wild relatives of soybean in Europe.

In 2004, soybean was grown commercially in 93 countries (2), with a combined production of 206 million metric tonnes of soybeans (6). The major producers of soybean are United States of America, Brazil, Argentina and China. Together, these countries are responsible for almost 90% of the total soybean production (6). About 60% of the total soybean production is genetically modified soybean (7). Within the European Union, soybean is grown on a small scale in Austria, Czech Republic, France, Germany, Greece, Hungary, Italy, Slovakia, Slovenia and Spain (6). Approximately 64% of all soybeans produced in the European Union are grown in Italy. It should be noted that only non-gm soybean is grown in Europe.

Molecular and biological aspects of the GM-plant

Origin and function of the introduced genes

Soybean line A2704-12 is genetically modified by means of a particle acceleration method. Two copies of the *pat* gene, fragments of the *bla* gene and pUC19 sequences were inserted in A2704-12 soybean. The *pat* gene confers tolerance to herbicides containing the active ingredient glufosinate ammonium.

An overview of the introduced sequences is given below:

- Vector backbone (pUC19);
- Right border repeat, derived from *Agrobacterium tumefaciens*, octopine plasmid TiAch5, cis-acting element for T-DNA transfer;
- CAMV 35S, promotor derived from the *Cauliflower mosaic virus* (CaMV);
- Synthetic polylinker sequence, plasmid cloning site;
- *pat*, gene from *Streptomyces viridochromogenes*; confers resistance to glufosinate ammonium;
- T35S terminator, derived from the *Cauliflower mosaic virus* (CaMV);
- Sequence of pUC19 vector, including the polylinker, *ori* (bacterial origin of replication) and *bla* (coding sequence for ampicillin resistance).

Properties of the introduced genes conferring herbicide tolerance

The soybean line was genetically modified with a *pat* gene encoding phospinothricin acetyltransferase. As a result, tolerance was obtained to glufosinate ammonium containing herbicides. The *pat* gene is coding for the PAT protein, which catalyzes the conversion of L-phosphinothricine (L-PPT), the active part of the herbicide glufosinate ammonium, to N-acetyl-L-PPT. This results in survival of the plant in case the herbicide is applied. Applying glufosinate ammonium to non-GM plants results in a decline in glutamine and an increase in ammonia levels

by inhibiting glutamine synthetase activity. As a result photosynthesis will stop and the plant dies within a couple of days (8).

Molecular analysis

The complete vector backbone is inserted in the plant, including polylinker sequences, the origin of replication and *beta-lactamase* (*bla*) gene sequences. Hybridization analysis indicate that two copies of the *pat* gene are inserted into the plant genome. A complete 957 base pair (bp) PvuI fragment of the vector is inserted between the two *pat* gene cassettes in an inverted orientation, as compared to the transforming plasmid configuration. The *bla* gene is not functional in the plant. The inserted plasmid construct was digested with a restriction enzyme (PvuI), disrupting the *bla* gene, prior to the transformation. Both integrated parts of the *bla* gene do not constitute an intact *bla* gene as the 5' *bla* sequences are integrated in an inverted orientation. This was confirmed by a Northern blot analysis performed on different tissues, employing a *bla* DNA fragment as a probe. COGEM is of the opinion that the presence of the pUC 19 vector sequences in the transgenic soybean poses no risks to human health and the environment.

Analysis of the flanking regions of the A2704-12 insert demonstrates that both the 5' and 3' flanking sequences are of soybean origin. However, the 5' flanking sequence of the insert appears to be chloroplast genomic DNA. Most likely the chloroplast sequences were co-integrated during the transformation process. PCR-analysis, using primers based upon the known chloroplast sequences and a primer located in the 35S promoter of the insert, revealed that the integrated chloroplast DNA is 2510 to 2718 nucleotides in length. The applicant determined the nucleotide sequence of the largest obtained PCR fragment. This way the sequence was determined of 2466 nucleotides of chloroplast origin.

The applicant sequenced a PCR-fragment obtained with primers based upon chloroplast sequences. Therefore, the obtained sequence neither comprises the entire co-integrated chloroplast DNA sequences nor does it extend into the 5’ flanking nuclear genomic sequences of the transgenic soybean plant. Since no data were provided on the actual 5’integration site, it can not be completely excluded that upstream of the chloroplast sequences other sequences were integrated. Moreover, it is possible that the integration of the chloroplast sequence or other putative sequences results in the rise of new chimeric open reading frames (ORFs) at the insertion site by fusion of ORFs of transgenic origin and plant origin.

The COGEM considers it important that the entire nucleotide sequence of the chloroplast DNA is determined and that the sequence analysis is extended into the 5’ nuclear genomic plant sequence. The COGEM points out that this is technically feasible, e.g. by genome walking in the untransformed soybean line from the 3' flanking sequence to the 5' flanking sequence, followed by primer design in the 5' flanking region and amplification and sequencing of the fragment in the transformed line between the 5' flanking region and the intended insertion. Furthermore, the obtained sequence data should be analyzed for the presence of new ORFs. The COGEM is of the

opinion that the applicants have to provide a more detailed study of the 5' flanking sequence of the insert and chloroplast DNA.

Advice

The present application concerns the import, processing and feed and food use of a soybean line. Cultivation of the present line is not part of the application. The risk assessment therefore focuses on the accidental spillage of soybean and the molecular analysis of this event. As stated above, soybean growth is very sensitive to temperature. A reasonably high temperature is required in all stages of development. The Dutch climate is not optimal for growing soybean. During the warmest months (April to October), the average temperature is around 16ºC (10). Moreover, the fact that the Netherlands are known for their frost periods in winter, makes it impossible for soybean to survive and to establish itself in the Netherlands.

At the moment soybean is grown in Austria, Czech Republic, France, Germany, Greece, Hungary, Italy, Slovakia, Slovenia and Spain (6) However, it is not to be expected that the spillage of soybeans can lead to the establishment of feral populations. Modern soybean cultivars do not possess any of the attributes commonly associated with problematic weeds and there are no reasons to assume that the inserted genes will increase the potential of the soybean to run wild. Furthermore, establishment of feral populations in soybean producing European countries is never observed. Even if climatic conditions will change in the Netherlands, the COGEM is of the opinion that there is a negligible risk of soybean to establish in the Netherlands, due to the fact that soybean is a short-day plant.

In view of the above, COGEM is of the opinion that there is a negligible risk that incidental spillage of soybean will lead to the spread of soybean within the European Union. Furthermore, there are no wild relatives of soybean present in Europe and therefore outcrossing with wild relatives is impossible.

A general surveillance plan is supplied by the applicant. General surveillance will be performed by either selected networks or organizations and/or specific company monitoring programs. However, it is unclear how the results of the monitoring are reported back to the applicant. Furthermore, the applicant makes a distinction between reporting direct and indirect effects in the monitoring plan. According to the applicant direct effects will be reported annually and indirect effects only at the stage of re-evaluation or at the end of a given consent. COGEM is of the opinion that the applicant should report both direct and indirect effects annually.

There is lack of information in the molecular analysis. The applicant does not provide sufficient information concerning the 5' flanking region of the insert. Chloroplast DNA is co-integrated with the insert. The applicant provides sequence data of the approximately 2500 bp chloroplast DNA in the flanking region, but does not show sequence data that extend into the nuclear genome of the parent plant. COGEM is of the opinion that these data are a prerequisite to exclude the potential for insertional events to produce any novel chimeric proteins.

In view of the deficiencies in the molecular analysis COGEM advises negatively on this application for the commercial import and processing for use in feed and food of a genetically modified soybean event A2704-12.

References

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