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Aan de minister van
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Ordening en Milieubeheer
Mevrouw dr. J.M. Cramer
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DATUM 19 december 2007
KENMERK CGM/071219-03
ONDERWERP Concept advies import en verwerking van soja 305423 (EFSA/GMO/NL/2007/45)

Geachte mevrouw Cramer,

Naar aanleiding van de adviesvraag betreffende het dossier EFSA/GMO/NL/2007/45, getiteld “Application for the authorization of genetically modified 305423 soybean and derived food and feed in accordance with Regulation (EC) No 1829/2003” voor de import en verwerking van genetisch gemodificeerde soja door Pioneer Hi-Bred International Inc. adviseert de COGEM als volgt:

Samenvatting:
De COGEM is gevraagd te adviseren over de toelating van genetisch gemodificeerde soja voor import en verwerking. Deze gg-soja bevat een *gm-fad2-1* gen fragment en een *gm-hra* gen resulterend in respectievelijk een verhoogd vetzuurgehalte en tolerantie voor acetolactaat synthase remmende herbiciden.
Teelt van soja en overwintering van sojabonen is in Nederland niet mogelijk. Soja beschikt niet over eigenschappen voor verwildering en er zijn geen redenen om aan te nemen dat de geïntroduceerde genen het verwilderingspotentieel vergroten. Bovendien zijn er in Europa geen wilde verwanten van soja aanwezig zodat uitkruising niet mogelijk is. De COGEM acht de kans op ecologische risico’s van deze genetisch gemodificeerde soja daarmee verwaarloosbaar klein. De COGEM is echter van mening dat de moleculaire karakterisering van de sojalijn onvolledig is. Een volledige moleculaire karakterisering is van belang bij de beoordeling door de COGEM of er nadelige effecten kunnen optreden bij incidentele consumptie en/of vraat. Echter, de voedingsproeven met kippen geven hiervoor geen aanwijzingen.
De COGEM is derhalve van mening dat de risico’s voor mens en milieu bij import van soja 305423 verwaarloosbaar klein zijn.
De COGEM realiseert zich dat ook andere instanties de voedsel- en veevoederveiligheid van deze sojalijn beoordelen. Het COGEM advies is afgegeven met de wetenschap dat een definitieve beslissing over dit dossier genomen zal worden op basis van een afweging van alle aspecten. Daarnaast is de COGEM van mening dat gezien de discussie omtrent de impact van de moleculaire karakterisering op de milieu-risicoanalyse dit onderwerp bediscussieert dient te worden binnen een Europese context. Dit ten einde te komen tot een gemeenschappelijke visie aangaande de minimale vereisten voor een moleculaire karakterisering.

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. D.C.M. Glandorf
Dr. I. van der Leij

Import and processing of herbicide tolerant soybean 305423

COGEM advice CGM/071219-03

Summary

The present application by Pioneer Hi-Bred International Inc. of file EFSA/GMO/NL/2007/45, 'Application for the authorization of genetically modified 305423 soybean and derived food and feed in accordance with Regulation (EC) No 1829/2003' concerns the import and processing for use in feed and food of a genetically modified soybean line. Cultivation is not part of this application.

The recombinant soybean line is genetically modified by insertion of a gm-fad2-1 gene fragment resulting in a high oleic phenotype. Soybean 305423 also contains the gm-hra gene conferring tolerance to certain herbicides.

In Europe, wild relatives of soybean are not 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 gene would increase the potential of the soybean to run wild. Furthermore, establishment of feral populations in soybean producing countries has never been observed. Survival of soybean is not possible in the North-Western European climate. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will not pose a risk to the environment.

COGEM points out that the molecular analysis of soybean line 305423 is incomplete. Therefore, it cannot be excluded that new open reading frames with potential adverse effects were created due to the insertion. However, COGEM is of the opinion that, based on the results of the poultry feeding study, it can be concluded that no harmful effects are caused by incidental consumption of 305423 soybean. Therefore, COGEM is of the opinion that the risks for import of soybean 305423 are negligible in case of incidental consumption.

In consideration of these aspects and because soybean cannot survive or establish itself in the Netherlands, COGEM is of the opinion that the import of soybean 305423 poses a negligible risk to humans and the environment.

COGEM realizes that also other authorities and advisory bodies are involved in this dossier which have a primary role in the assessment of the food and feed safety. This COGEM advice is given in the understanding that a final decision by the authorities will be based on an integrative interpretation of all aspects concerned.

Furthermore, in view of the different aspects which are related to the impact of the molecular characterization, COGEM is of the opinion that this topic should be discussed within a European context in order to obtain a common view on the minimum requirements as far as the molecular characterization is concerned.

Introduction

The present application by Pioneer Hi-Bred International Inc., file EFSA/GMO/NL/2007/45, concerns the import and processing of soybean line 305423 use in feed and food. This line contains and expresses the *gm-fad2-1* gene, resulting in a high oleic phenotype. Furthermore, the *gm-hra* gene is expressed which confers tolerance to acetolactate synthase (ALS)-inhibiting herbicides.

Previous COGEM advices

In 1995 and 2006, COGEM advised positively on import, processing and cultivation of soybean line 40-3-2, which contains the *cp4 epsps* gene (1,2). In 2006, COGEM advised negatively on soybean A2704-12 as a result of flaws in the molecular characterization (3). In 2007, COGEM advised negatively on import and processing of *cp4 epsps* expressing soybean line MON89788 due to an incomplete molecular analysis (4).

Aspects of the crop

Soybean (*Glycine max*) is a member of the genus *Glycine* and belongs to the *Fabaceae* (*Leguminosae*) family and is grown from equatorial to temperate zones. The optimum temperature for soybean growth is between 25 °C and 30 °C. Depending on cultivar and climate, the growth period can range from 65 to 150 days. The seed will germinate when the soil temperature reaches 10 °C and it emerges in 5-7 days under favorable conditions (6). The crop starts to flower 25 to 150 days after sowing, depending on the day length, temperature and cultivar. Flowering can take 1-15 days; pod formation 7-15 days; seed filling 11-20 days and ripening to harvest 7-15 days (5). Soybean is a quantitative short-day plant and hence, flowers more quickly under short days. Temperatures below 21 °C and above 32 °C can reduce floral initiation and pod set. Soybean is susceptible to frost damage and does not survive freezing winter conditions (6).

Soybean is considered 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 (6). Therefore, insect-born exportation of pollen is limited and the cross-pollination rate of soybean is less than 1% (5). There are no wild relatives of soybean in Europe.

Soybean is only propagated by seed. Animal transportation is not encouraged by the morphology of the seedpod or seeds (6), but dispersal of seeds may occur by humans during transport, sowing or harvest. The soybean plant is not weedy in character (6). Cultivated soybean rarely displays any dormancy characteristics (6) and seeds of cultivated soybean survive poorly in soil (7). Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers (6). In addition, volunteers can easily be controlled mechanically or chemically (6).

In 2004, soybean was grown commercially in 72 countries, with a total production of 206 million metric tons (8). The major producers of soybean are the United States of America, Brazil, Argentina and China. These four countries are responsible for 90% of the total soybean production (8). In 2005, 60% of the global soybean area was genetically modified (9). In Europe, only non-genetically modified soybean is grown.

General surveillance plan

A general surveillance plan is supplied by the applicant. As stated before, COGEM is of the opinion that the applicant should describe in more detail how the general surveillance will be organized and should indicate which organizations are involved. In addition, the applicant should ascertain that information on eventual adverse effects is indeed obtained. Furthermore, direct and indirect effects should be reported annually.

However, because soybean can not survive in the North-Western European climate, a general surveillance plan is in this specific case of less importance for the situation in the Netherlands.

Molecular characterization

Origin and function of the introduced genetic elements

Soybean line 305423 was genetically modified by means of particle co-bombardment.

The introduced sequences are:

- *KTi3* promoter,
  Soybean Kunitz trypsin inhibitor gene 3 (KTi3) promoter;
- *Gm fad2-1* gene fragment
  597 bp fragment of the coding region of the microsomal omega-6 desaturase gene 1 from *Glycine max* (FAD2-1 gene);
- *KTi3 t*erminator,
  Soybean Kunitz trypsin inhibitor gene 3 (KTi3) terminator;
- SAMS promoter,
  S-adenosyl-L-methionine synthetase (SAMS) constitutive promoter from *Glycine max*;
- SAMS 5'-UTR,
  5'-untranslated region of the SAMS gene from *G. max*;
- SAMS intron,
  intron within the SAMS 5'-UTR from *G. max*;
- *gm-hra* gene,
  modified endogenous acetolactate synthase (*als*) gene derived from *G. max*;

- *als* terminator,
  terminator region from soybean endogenous acetolactate synthase gene.

Properties of the introduced genes

Soybean line 305423 was genetically modified with a *gm fad2-1* gene fragment. The gene fragment is part of the coding region of the soybean endogenous FAD2-1 gene and does not code for a functional protein. Transcription of the *gm-fad2-1* gene fragment in 305423 soybean seeds acts to suppress transcription of endogenous omega-6-desaturase, resulting in a high oleic phenotype.

Besides *gm-fad2-1*, the *gm-hra* gene was introduced. The *gm-hra* gene is an optimized form of the endogenous acetolacte synthase (*ALS)* gene. The enzyme catalyzes the first step in the biosynthesis of the essential amino acids valine, leucine and isoleucine. The mode of action of a couple of herbicides is based on the interruption of this enzyme. Interruption will result in disruption of the formation of the amino acids causing the plant to die. Mutations in the *als* gene have resulted in an increased tolerance for these herbicides because the affinity between enzyme and herbicide is decreased in this way.

Molecular analysis

In COGEM's opinion, the applicant has proven by Southern blot that a single intact copy of the restriction fragment is present in 305423. However, sequence analysis of the inserted DNA in 305423 soybean confirms that multiple intact and truncated copies of the PHP1934A have been inserted into 305423 soybean. In total, eight copies of the KTi3 promotor, seven copies of the *gm-fad2-1* gene fragment and five copies of the KTi3 terminator have been inserted. A single intact copy of fragment PHP17752A has been inserted into 305423 soybean. Despite of a small non-functional fragment of the plasmid backbone, Southern Blot analysis confirms the absence of all functional elements from the plasmid backbone.

Although DNA sequence analysis confirms that the flanking regions of the insert consist of genomic soybean DNA, PCR amplification data to validate these results are lacking. Therefore, COGEM is of the opinion that it cannot be concluded that no DNA rearrangements have taken place.

The junctions between the T-DNA and its flanking regions were examined for the presence of potential novel open reading frames (ORFs). The applicant identified a total of 84 possible ORF's in the 5' and 3' junction regions of soybean genomic sequences and the inserted DNA as well as within the four insertion regions in 305423 soybean.

The nucleotide sequences in all six frames of the junction were translated into amino acid sequences. Novel ORF's containing both start and stop codons were screened for the presence of novel fusion sequences. Twenty-seven of 84 possible ORF's across the novel

junctions were containing an ATG start codon and were therefore considered translationally competent. After comparison to the FARRP7 allergen dataset, the applicant concluded that the deduced amino acid sequences, ranging from 9 to 235 residues in length, had no significant homology to any known or putative toxins or allergens.

The applicant defined an ORF as a region that initiates with an ATG codon and ends with any of the three stop codons TAA, TAG or TGA. COGEM notes that translation may initiate with other codons or that an ORF, which does not start with an ATG, becomes part of a longer ORF by the process of pre-mRNA splicing. Therefore, COGEM is of the opinion that also ORFs that could initiate with other start codons should be examined.

Molecular characterization and the environmental risk analysis

In the past, COGEM has advised negatively on several applications due to incomplete molecular characterization. COGEM is aware that other advisory committees, such as EFSA, appear to follow a less stringent approach regarding the requirements of molecular characterization. This was one of the reasons that COGEM recently discussed this issue in more detail in one of its meetings. The preliminary outcome of this discussion is that a full characterization of the insert is needed, according to COGEM. The degree to which also the flanking regions of the insert should be analyzed, to exclude adverse effects as much as possible, is under debate. It should be noted that in most cases these potential risks will be related to incidental consumption rather than ecological aspects.

In addition, COGEM points out that a better molecular characterization is also of importance in view of transparency. Therefore, COGEM urges applicants to provide a better molecular characterization as indicated above.

Advice

COGEM has been asked to advice on import and processing for use in feed and food of soybean line 305423.

The Dutch climate does not have the proper conditions for soybean growth. The optimum temperature for soybean growth is 25 °C to 30 °C. In the Netherlands, 16.8 °C was the average summer temperature from 1971 to 2006, and 18.6 °C was the average temperature of the three warmest summers since 1901 (11). In addition, soybean is susceptible to frost damage and does not survive freezing winter conditions. In the Netherlands frost is common. During the winter, on average 38 days are measured with a minimum temperature below 0 °C (11). In conclusion, the Dutch climate prohibits the survival and establishment of soybean plants. Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days

for fructification. In view of the above, in COGEM's opinion it is unlikely that soybean could establish itself in the Netherlands even if the climate changes.

Modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds and there is no reason to assume that expression of the introduced *gm-hra* gene and the *fad2-1* gene fragment increase the potential of soybean to run wild. In addition, establishment of feral soybean populations in European countries has never been observed. COGEM is of the opinion that incidental spillage of soybean is very unlikely to lead to the spread of soybean within the European Union. In addition, wild relatives of soybean are not present in Europe and therefore introgression of the inserted genes into wild relatives cannot occur.

In COGEM's view, the molecular analysis of soybean 305423 is incomplete. Not all putative ORFs were screened for potential allergenicity or toxicity but only those considered translationally competent (by the presence of an ATG startcodon) by the applicant. Since translation can be initiated by other codons which are not included in the analysis, it cannot be excluded that new open reading frames were created due to the insertion. If new open reading frames were created, these could theoretically give rise to proteins with potential adverse effects like toxicity or allergenicity.

However, feed and toxicity studies could indicate if indeed any proteins with adverse effects like toxins are formed. The applicant compared the growth performance and carcass yield of 60 male and 60 female broiler chickens in a 42-day feeding study. Broiler chickens were fed with diets containing processed fractions (meal, hulls, and oil) from 305423 soybean during this period and were compared with a control group of broiler chickens that were fed processed fractions from non-transgenic soybeans. COGEM is of the opinion that on basis of this study in poultry no harmful effects caused by incidental consumption of 305423 soybean can be expected.

In consideration of these aspects and because soybean cannot survive or establish itself in the Netherlands, COGEM is of the opinion that the import of soybean 305423 poses a negligible risk to humans and the environment.

COGEM realizes that also other authorities and advisory bodies are involved in this dossier which have a primary role in the assessment of the food and feed safety. This COGEM advice is given in the understanding that a final decision by the authorities will be based on an integral interpretation of all aspects concerned. Furthermore, COGEM is of the opinion, in view of the different concerns related to an adequate molecular characterization, that this topic should be discussed within a European context in order to obtain a common view on the minimum requirements for the molecular characterization.

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