Import and processing of herbicide tolerant soybean 356043

COGEM advice CGM/071129-01

Summary

The present application by Pioneer Hi-Bred International Inc. of file EFSA/GMO/NL/2007/43, 'Application for the authorization of genetically modified 356043 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 the gm-hra and gat4601 gene. As a result, 356043 is tolerant 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. In addition, 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. Moreover, survival of soybean is not possible in the North-Western European climate. Survival and establishment of soybean volunteers in the wild has never been reported in Europe. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will not pose a risk to the environment.

However, COGEM points out that the molecular analysis of soybean line 356043 is incomplete. Therefore, 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 potential toxic or allergenic products. Feeding and toxicity studies could rule out if indeed allergens or toxins are formed. COGEM has some reservations about the quality of the studies in the dossier. Therefore, she can not advise positively on this application at the moment. 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/2007/43, concerns the import and processing of soybean line 356043 use in feed and food. This line contains and expresses the *gat4601* gene, which confers tolerance to glyphosate based herbicides. Besides, the gene *gm-hra* is expressed which confers tolerance to acetolactate synthase (ALS)-inhibiting herbicides.

According to the applicant, notification concerning all uses of 356043 soybean, including cultivation, have been submitted in the United States of America, Canada and Mexico.

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 also negatively on import and processing of soybean line MON89788 (4). This soybean line expresses the *cp4epsps* gene. The molecular analysis of MON89788 was considered incomplete.

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 356043 was genetically modified by means of particle acceleration. The introduced sequences are:

- SCP1 promoter,
  synthetic constitutive promoter consisting of sequences of the CaMV 35S promoter and the Rsyn7-Syn II core consensus promoter;
- TMV omega 5'-UTR,
  5' non translated leader gene of *Tobacco mosaic virus* (TMV), which enhances translation;
- *gat4601* gene,
  synthetic gene originated from *Bacillus licheniformis*, optimized for glyphosate acetyltransferase production;
- *pin*II terminator,
  terminator region from *Solanum tuberosum*;
- 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 356043 was genetically modified with a synthetic *gat4601* gene. The gene is based on glyphosate acetyltransferase protein sequences from *Bacillus licheniformis*. To synthesize the *gat4601* gene, three distinct alleles of the *gat* gene were isolated from three different strains of *B. licheniformis*. In order to develop an enzyme useful for the introduction of glyphosate tolerance into the genetically modified soybean, DNA shuffling was applied on the three alleles. In this way, glyphosate acetyltransferase activity of the native GAT enzymes from *B. licheniformis* could be optimized.

Tolerance of plants against herbicides is commonly achieved through metabolic detoxification of herbicides by native plant or transgene-encoded enzymes. The advantage of glyphosate detoxification is the removal of herbicidal residue, which may result in more robust tolerance and allow spraying during reproductive development (10). Detoxification could be achieved by hydrolysis, acetylation or oxidative cleavage of the herbicide. In this case, the herbicide glyphosate is detoxified by acetylation by means of the glyphosate N-acetyltransferase enzyme encoded by the *gat4601* gene.

Besides *gat4601*, the gene *gm-hra* 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 356043. Results obtained by PCR amplification and DNA sequence analysis confirm that the flanking regions of the insert consist of genomic soybean DNA. It was not clear if rearrangements have taken place around the

insertion site. A PCR analysis of the non-transgenic line could resolve whether the 5' and 3'flanking regions are contiguous in the non-transgenic soybean genome.

The junction between the T-DNA and its flanking regions was examined for the presence of potential novel open reading frames (ORFs). The nucleotide sequences in all six frames of the junction were translated into amino acid sequences. The applicant stated that only one of the 12 possible ORFs contained the necessary start and stop codons for potential translation. This peptide was compared to both a dataset of known and putative allergens and to publicly available protein datasets. Results from the comparisons showed that the peptide did not match to known or putative allergens or toxins.

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 although most ORFs initiate with an ATG codon, translation may initiate with other codons or that an ORF, which does not begin 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 discussed the issue in more detail. COGEM is of the opinion that a full characterization of the insert is needed. The degree to which also the flanking regions should be analyzed, to exclude adverse effects as much as possible, is under debate. 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.

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.

Advice

COGEM has been asked to advice on import and processing for use in feed and food of soybean line 356043.

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* and *gat4601* genes 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, it is sufficiently proven that only one copy of the insert and its genetic elements are present in 356043. However, it was not made clear if any genomic rearrangements occurred during the transformation. In addition, not all putative ORFs were screened for potential allergenicity or toxicity. Feed and toxicity studies could indicate if indeed any allergens or toxins are formed. However, COGEM has reservations regarding the quality of the toxicity studies presented. On basis of the data provided COGEM can not make a substantiated risk analysis regarding any potential adverse effects related to incidental consumption. As a result, she can not advise positively on this application at the moment.

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.

References

1. COGEM (1995). Import and processing of glyphosate tolerant soybean 40-3-2. COGEM advice CGM/950626-16

2. COGEM (2006). Cultivation of glyphosate tolerant soybean 40-3-2. COGEM advice CGM/061128-01
3. COGEM (2006). Import and processing of herbicide tolerant soybean A2704-12. COGEM advice CGM/060410-04
4. COGEM (2007). Import and processing of glyphosate tolerant soybean MON 89788. COGEM advice CGM/070807-01
5. Crop Protection Compendium (2004). *Glycine max* (soybean). CD-ROM edition, ©Cab International 2004, Nosworthy way, Wallingford, UK
6. OECD (2000). Consensus document on the biology of *Glycine max* (L.) Merr. (Soybean)
7. OECD (1993). Traditional crop breeding practices: An historical review to serve as baseline for assessing the role of modern biotechnology
8. FAO (2006). Statistical yearbook 2005-2006, pp-83-86
9. Gómez-Barbero M and Rodríguez-Cerezo E (2007). GM crops in EU agriculture. European Commission, Directorate-General Joint Research Centre, Institute for Prospective Technological Studies (Seville)
10. Castle LA, Siehl DL, Gorton R *et al*. (2004). Discovery and directed evolution of a glyphosate tolerance gene. Science 304: 1151-1154
11. Koninklijk Nederlands Meteorologisch Instituut (KNMI): www.knmi.nl/klimatologie/maand_en_seizoensoverzichten (24 July 2007)