Import and processing of glyphosate tolerant soybean MON 89788

COGEM advice CGM/070807-01

Summary

The present application by Monsanto Europe S.A. of file EFSA/GMO/NL/2006/36, 'Glyfosate-tolerant soybean transformation event MON 89788', 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 cp4 epsps gene. As a result, MON 89788 is tolerant to herbicides containing the active ingredient glyphosate.

Recently, MON 89788 has been approved for use as food in the United States of America and Canada and for use as feed in the United States of America.

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 probably not pose a risk to man and the environment.

However, COGEM points out that the molecular analysis of soybean line MON 89788 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 give rise to potential toxic or allergenic products. Considering the requirements of EU Directive 2001/18, COGEM is of the opinion that the molecular characterization was insufficiently provided. In view of the shortcomings of the molecular analyses, COGEM cannot advice positively on the application for import and processing of soybean line MON 89788. Furthermore, COGEM has some comments on the general surveillance plan.

Introduction

The present application by Monsanto Europe S.A., file EFSA/GMO/NL/2006/36, concerns the import and processing of soybean line MON 89788 for use in feed and food. This line contains and expresses the gene *cp4 epsps*, which confers tolerance to glyphosate based herbicides.

In 2007, MON 89788 soybean has been authorized for use as food and/or feed in the United States of America and for use as food in Canada (1).

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 (2, 3). In 2006, COGEM advised negatively on import and processing of soybean line A2704-12 (4). This soybean line expresses the *pat* gene, which confers tolerance to glufosinate ammonium containing herbicides. The molecular analysis of A2704-12, as mentioned in EU Directive 2001/18, was considered incomplete because the required information on the 5' flanking region of the insert was not provided.

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 tonnes (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.

Molecular characterization

Origin and function of the introduced genetic elements

Soybean line MON 89788 was genetically modified by *Agrobacterium tumefaciens* mediated transformation. The introduced sequences are:

- Right border,
  DNA region from *A. tumefaciens* used for transfer of T-DNA;
- *FMV/Tsf1* promoter,
  chimeric promoter consisting of enhancer sequences from the 35S promoter of the *Figwort mosaic virus* and the promoter from the *Tsf1* gene of *Arabidopsis thaliana*, which encodes elongation factor EF-1alpha;
- *Tsf1* leader and intron,
  5' non translated leader (exon 1) and intron from the *Tsf1* gene of *A. thaliana*, which encodes elongation factor EF-1alpha;
- *CTP2* targeting sequence,
  chloroplast transit peptide from the *ShkG* gene of *A. thaliana,* which encodes the CP4 EPSPS protein;
- *CP4 EPSPS* coding sequence,
  codon optimized sequence of the *aroA (epsps)* gene from the *Agrobacterium* sp. strain CP4, which encodes the CP4 EPSPS protein;
- *E9* termination and polyadenylation signal sequence,
  3' non translated sequence from the ribulose-1,5,-bisphosphate carboxylase small subunit (*RbcS2*) E9 gene of pea (*Pisum sativum*);
- Left border,
  DNA region from *A. tumefaciens* used for transfer of T-DNA.

Properties of the introduced gene

Soybean line MON 89788 was genetically modified with a functional *cp4 epsps* gene, which encodes for the CP4 EPSPS protein. The *FMV/Tsf1* promoter drives constitutive expression of the CP4 EPSPS protein.

EPSPS is an enzyme involved in the biosynthesis of aromatic amino acids. Glyphosate inhibits EPSPS, which results in a lack of amino acids essential for growth and development of plants. The majority of EPSPS enzymes are sensitive to glyphosate. The CP4 EPSPS protein, however, is not inhibited by glyphosate (10). MON 89788 expresses the CP4 EPSPS protein and is therefore tolerant to glyphosate.

Molecular analysis

In COGEM's opinion, the applicant has proven by hybridization analysis that the backbone of the plasmid used for *A. tumefaciens* mediated transformation is absent in MON 89788. In addition, the applicant demonstrated by hybridization analyses that MON 89788 contains a single copy of the insert and its elements. Results obtained by PCR amplification and DNA sequence analyses confirmed that an intact insert was integrated. Furthermore, hybridization analyses showed that the insert is stable over several generations.

However, the applicant does not provide evidence for the statement that the flanking regions of the insert are soybean genomic DNA. The results of bioinformatic analyses on the DNA sequences are not shown and because the sequence is confidential and may not be subjected to BLAST analyses the applicant's conclusions cannot be confirmed. In addition, the conclusion that the insert was not inserted into an active gene was not substantiated with data.

Furthermore, COGEM is of the opinion that it has not sufficiently been proven that the complete chromosomal integration site was characterized. The presence of soybean genomic DNA in the flanking regions could also be explained by co-integration of soybean DNA from other parts of the genome. Comparing the results from PCR analyses on MON 89788 and a non-transgenic soybean line would indicate whether the 5' and 3' flanking regions are contiguous in the non-transgenic soybean genome.

In addition, not all hypothetical polypeptides present in the inserted T-DNA border regions were assessed for possible similarities to allergens or toxins. Although the applicant assessed hypothetical polypeptides in the regions spanning the junctions of the soybean genomic DNA and the inserted DNA, these analyses did not include the entire inserted T-DNA border regions. COGEM is of the opinion that all hypothetical polypeptides of the entire inserted T-DNA border regions should be assessed for possible similarities to allergens and toxins.

In view of the above, COGEM is of the opinion that because of the crop's characteristics environmental risks of the import of MON 89788 are probably negligible. However, this opinion cannot be substantiated because the molecular characterization is incomplete. Therefore, authorization should not be granted until the missing data has been provided.

General surveillance plan

A general surveillance plan is supplied by the applicant. General surveillance will be performed either by selected networks and/or specific company stewardship programs. The permit holder will request key stakeholders and networks to participate and asks them to be informed if any unanticipated adverse effects occur. However, it is unclear

how these adverse effects are monitored if key stakeholders and networks do not assist. In COGEM's opinion the permit holder should ascertain that information on adverse effects is obtained even if key stakeholders and networks do not participate.

In addition, 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, whereas indirect effects will only be reported at the stage of re-evaluation or at the end of a given permit. As stated before, in COGEM's opinion the applicant should report both direct and indirect effects annually.

Advice

COGEM has been asked to advice on import and processing for use in feed and food of soybean line MON 89788.

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 *cp4 epsps* gene increases 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 *cp4 epsps* gene into wild relatives cannot occur.

In COGEM's view, it is sufficiently proven that backbone sequences are absent and that only one copy of the insert and its genetic elements are present in MON 89788. However, the molecular analyses are incomplete. The conclusion that the flanking regions are soybean genomic DNA is not substantiated with data. In addition, it has not been sufficiently proven that the complete chromosomal integration site was characterized. Furthermore, not all hypothetical polypeptides present in the inserted T-DNA border regions were assessed for possible similarities to allergens and toxins.

Because of the incomplete molecular analyses it cannot be excluded that new open reading frames were created due to the insertion. If new open reading frames were created, these could give rise to potential toxic or allergenic products.

COGEM is of the opinion that the risks to man and the environment of the import of MON 89788 are probably negligible. However, in view of the lacking data concerning the molecular characterization as mentioned in EU Directive 2001/18, this opinion can not be sufficiently substantiated. Therefore, COGEM cannot advice positively on the application for import and processing of soybean line MON89788.

References

1. Agbios GM database product description: www.agbios.com (19 July 2007)
2. COGEM (1995). Import and processing of glyphosate tolerant soybean 40-3-2 (CGM/950626-16)
3. COGEM (2006). Cultivation of glyphosate tolerant soybean 40-3-2 (CGM/061128-01)
4. COGEM (2006). Import and processing of herbicide tolerant soybean A2704-12 (CGM/060410-04)
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. Funke T, Han H, Healy-Fried ML *et al.* (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. Proceedings of the National Academy of Sciences of the United States of America: 103, 13010-13015
11. Koninklijk Nederlands Meteorologisch Instituut (KNMI): www.knmi.nl/klimatologie/maand_en_seizoensoverzichten (24 July 2007)