Import and processing of soybean line A5547-127

COGEM advice CGM/080918-02

Summary

The present notification by Bayer CropScience AG concerns the import and processing for use in feed and food of soybean line A5547-127. Cultivation is not part of this application.

Due to the presence of a pat gene, A5547-127 is tolerant to glufosinate-ammonium containing 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, survival of soybean is not possible in the North-Western European climate. Establishment of feral soybean populations has never been observed in European countries. There is no reason to assume that the inserted gene would introduce or increase the potential for soybean to establish feral populations. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will not pose a risk to the environment.

In consideration of these aspects, COGEM is of the opinion that the import and processing of soybean line A5547-127 poses a negligible risk to the environment.

Introduction

The present application by Bayer CropScience AG, file EFSA/GMO/NL/2008/52, concerns the import and processing of soybean A5547-127 for use in feed and food. This line expresses the *pat* gene, which confers tolerance to glufosinate-ammonium containing herbicides. In 1998, soybean A5547-127 was authorized for commercial import, processing and cultivation in the United States of America (1). However, it has not been commercialized (2).

Previous COGEM advices

In 2007, COGEM advised positively on import and processing of soybean A2704-12. This line also expresses the *pat* gene and is tolerant for herbicides containing glufosinate-ammonium (3).

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. Due to the meteorological and geographical conditions cultivation of soybean is impossible in the

Netherlands. The optimum temperature for soybean growth is between 25°C and 30°C. In the Netherlands, 16.8°C was the average summer temperature from 1971 to 2008. The average temperature of the three warmest summers since 1901 was 18.6°C (4). In addition, soybean is susceptible to frost damage and does not survive freezing. In the Netherlands frost is common; during winter on average 38 days are measured with a minimum temperature below 0 °C (4). Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days for fructification.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1% (5). Cross-pollination occurs by insects. 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 (5). Hybridization with other species is not possible because there are no wild relatives of soybean in Europe.

The soybean plant is not weedy in character (6). Cultivated soybean rarely displays dormancy (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 are easily controlled mechanically or chemically (6). Establishment of feral soybean populations has never been observed in European countries.

Molecular characterization

Soybean line A5547-127 was genetically modified by particle bombardment with plasmid pB2/35SAcK (derivative of plasmid pUC19). This plasmid contains the following genetic elements:

- Vector backbone pUC19
- Right border fragment, derived from *Agrobacterium tumefaciens* octopine plasmid TiAch5, *cis*-acting element for T-DNA transfer
- P35S, promoter derived from the *Cauliflower mosaic virus* (CaMV)
- Synthetic polylinker derived sequence, plasmid cloning site
- *pat*, synthetic gene; amino acid sequence from *Streptomyces viridochromogenes*; confers resistance to glufosinate ammonium
- Synthetic polylinker derived sequence, plasmid cloning site
- T35S, terminator derived from CaMV
- Ori and *amp* (=*β-lactamase*; *bla*), sequence of vector pUC19, bacterial origin of replication and bacterial selection marker for ampicillin resistance

Properties of the introduced genes conferring herbicide tolerance

Soybean line A5547-127 is tolerant to herbicides containing glufosinate ammonium. In non-transgenic plants glufosinate ammonium inhibits the activity of glutamine synthetase, an enzyme necessary for the production of glutamine and for ammonia detoxification (8). The application of glufosinate ammonium leads to reduced glutamine and increased ammonia levels in non-transgenic plants (8). Photosynthesis is inhibited and eventually the plant dies (9). Soybean line A5547-127 expresses the *pat* gene, which encodes phosphinothricin-N-acetyl transferase (PAT). This protein acetylates L-phosphinothricin, the active isomer of glufosinate ammonium. The resulting compound N-acetyl-L-phosphinothricin does not inhibit the activity of glutamine synthetase (8). As a result A5547-127 is tolerant to L-phosphinothricin and thus to herbicides containing glufosinate ammonium.

Molecular analysis

In the opinion of COGEM, the molecular analysis of soybean line A5547-127 is adequately performed. The applicant showed by Southern blot, PCR and sequence analysis that a single copy of the *pat* gene cassette is integrated in the nuclear genome of soybean A5547-127.

Southern blot hybridization demonstrated that the backbone of plasmid pB2/35SAcK is partly integrated. The origin of replication of the plasmid is integrated in A5547-127 but is non-functional in plants.

Part of the *bla* gene (base pairs 861 to 438 of the *bla* gene) was located on the fragment which was used to insert the *pat* gene cassette by particle bombardment in the soybean genome. Moreover, the 5' end of the *bla* gene (base pairs 419 to 29 of the *bla* gene) co-integrated at the 5' end of the *pat* gene cassette. Both parts of the *bla* gene do not reconstitute a full-length *bla* gene. Northern blot analysis confirmed that the *bla* sequences are not expressed in the plant.

Southern blot analysis and Mendelian inheritance patterns demonstrated that the insert is genetically stable in three consecutive generations.

Furthermore, the 5' and 3' regions that flank the insert were amplified by PCR and sequenced. The obtained sequences were compared to soybean genomic DNA. This showed that the flanking sequences are of soybean origin and that no unintended insertion or deletion at the integration site has occurred. By BLAST analysis with sequences in databases it was shown that the sequence of the insertion site has similarity with mRNA/cDNA sequences of which the function is unknown. It is unlikely that an essential gene is disrupted by the insertion as there are no phenotypic differences reported between A5547-127 and untransformed soybean.

Insertion of the *pat* gene cassette and of the truncated 5' *bla* sequence resulted in three junction regions which potentially could give rise to new fusion proteins. The junctions were sequenced and analyzed for ORFs of 8 or more amino acids and starting with ATG. Eight putative ORFs were identified. One ORF could be expressed by a mRNA formed by the splicing of two exons, one spanning the 5' junction and one in the truncated 5' *bla* sequence. However, with Northern blot analysis no RNAs derived from the *bla* gene junction regions could be detected, indicating that these putative ORFs are not detectably expressed. Furthermore, none of the putative junction polypeptides showed homology with known toxins or allergens. In view of the above, the molecular analysis does not indicate that import and processing of soybean A5547-127 poses a risk to the environment.

Recently, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advice 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 and RIVM. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT, RIVM) was not known at the moment of the completion of this advice.

General surveillance plan

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

In the present application, a detailed general surveillance plan is provided to observe and register adverse effects of the import of A5547-127 timely. Following the initial placing on the market, the authorization holder will submit general surveillance reports on an annual basis for the duration of the authorization period.

Observations for unanticipated adverse effects will be monitored by existing systems which include the authorization holder and operators involved in the handling and use of viable A5547-127 soybean. Operators involved in the import, handling and processing of A5547-12 soybean inform the European trade associations (COCERAL, UNISTOCK and FEDIOL) of observed adverse effects. The trade associations report these effects to the authorization holder via the European Association of Bioindustries (EuropaBio) or directly to the authorization holder. EuropaBio is an association of members of the plant biotechnology industry which hosts a website containing information on approved

genetically modified plants subject to general surveillance. The website contains an e-mail address and a telephone number to exchange information on the plants. COGEM points out that to gather general surveillance data a questionnaire would be helpful. By placing such a list on the website, essential information on adverse effects can be collected in a more coherent and consistent manner.

As mentioned in previous advices, COGEM prefers independent organizations which have expertise on the environment and whose activities continue after the authorization period to be involved in general surveillance. In a previous advice on post-market monitoring, COGEM has outlined the standards that have to be met by a post-market monitoring system and has identified organizations which could be involved in post-market monitoring in the Netherlands (10).

Advice

COGEM has been asked to advice on import and processing for use in food and feed of soybean line A5547-127. Because cultivation is not part of the present application, the risk assessment focuses on the accidental spillage of soybean.

COGEM points out that the North-Western European climate prohibits survival and establishment of soybean. Furthermore, modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds and there is no reason to assume that presence and expression of the introduced gene increase the potential of soybean to establish feral populations. 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.

The molecular analysis of A5547-127 does not indicate that import and processing of this line would pose a risk to the environment.

In consideration of the aspects above, COGEM is of the opinion that import and processing of soybean A5547-127 poses negligible risks to the environment.

References

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