

# Import of genetically modified soybean DAS-68416-4 with two herbicide tolerance traits

## COGEM advice CGM/111114-02

### Summary

*The present application of Dow AgroSciences Europe (EFSA/GMO/NL/2011/91) concerns the import and processing for use in feed and food of genetically modified soybean DAS-68416-4. Cultivation is not part of this application.*

*Soybean line DAS-68416-4 was obtained by Agrobacterium tumefaciens mediated transformation of conventional soybean. The line expresses the aad-12 gene and the pat gene conferring tolerance to several herbicides.*

*In Europe, there are no wild relatives of soybean and therefore, hybridisation with other species is not possible. Soybean does not possess any of the attributes commonly associated with problematic weeds and establishment of feral soybean populations has never been observed in Europe. In addition soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers. COGEM is of the opinion that the environmental risk of spread of soybean DAS-68416-4 within the Netherlands due to incidental spillage of this soybean is negligible.*

*In the opinion of COGEM, the molecular analysis of soybean line DAS-68416-4 is sufficient. Although the general surveillance (GS) plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current general surveillance plan sufficient for import and processing of soybean line DAS-68416-4.*

*In conclusion, COGEM is of the opinion that import and processing of soybean line DAS-68416-4 poses a negligible risk to the environment. COGEM points out that a food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.*

### Introduction

The present notification EFSA/GMO/NL/2011/91 by Dow AgroSciences Europe concerns import and processing of the genetically modified soybean DAS-68416-4. This soybean line was produced by *A. tumefaciens* mediated transformation of conventional soybean and expresses the *aad-12* gene derived from *Delftia acidovorans* and the *pat* gene from *Streptomyces viridochromogenes*. These genes confer tolerance to several aryloxyalkanoate based and glufosinate-ammonium based herbicides.

### Previous COGEM advice

COGEM has not advised on plants with both herbicide tolerance traits before. However, in 2007 COGEM advised positively on import and processing of soybean A2704-12<sup>1</sup> and in 2008 on import and processing of soybean line A5547-127.<sup>2</sup> Both lines express the *pat* gene and are therefore tolerant to glufosinate-ammonium based herbicides.

In May 2011 COGEM advised positively on import and processing of the genetically modified maize line DAS-40728-9 which expresses the *aad-1* gene conferring tolerance to several aryloxyalkanoate based herbicides.<sup>3</sup>

### **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. The optimum temperature for soybean growth is between 25°C and 30°C. Soybean seeds will germinate when the soil temperature reaches 10°C and under favorable conditions a seedling will emerge in a 5-7 day period. Soybean is sensitive to frost and therefore does not survive freezing winter conditions.<sup>4</sup>

In the Netherlands, frost is common. During winter on average 38 days are measured with a minimum temperature below 0°C.<sup>5</sup> In the summer days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering. The Dutch climate is therefore not optimal for cultivation of soybean. However, field trials with a number of soybean varieties have shown that cultivation of soybean is possible.<sup>6,7</sup> Further improvement of these varieties may result in soybean varieties suited for commercial cultivation in the Netherlands.

The soybean plant is not weedy in character.<sup>4</sup> Cultivated soybean seeds rarely display dormancy and poorly survive in soil.<sup>8</sup> Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers.<sup>4</sup> In addition, volunteers are easily controlled mechanically or chemically.<sup>4</sup> COGEM is not aware of any reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.<sup>4</sup> The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower. In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.<sup>4</sup>

### **Molecular characterization**

DAS-68416-4 was developed by *Agrobacterium*-mediated transformation of the conventional soybean line Maverick using the disarmed *Agrobacterium* strain EHA101 carrying the binary vector pDAB4468. Glyphosate tolerance was used as a tool to select transformed plants. An overview of the T-DNA introduced in DAS-68416-4 is given below:

- T-DNA border B; 24 bp transferring DNA sequences.
- Intervening sequence; Sequence from Ti plasmid pTi15955.
- RB&-MAR; Matrix attachment region (MAR) from *Nicotiana tabacum* which potentially facilitates expression of the *aad-12* gene in the plant.
- Intervening sequence; Sequence from plasmid pENTR/D-TOPO and multiple cloning sites.
- AtUbi10; *Arabidopsis thaliana* polyubiquitin UBQ10 comprising the promoter, 5' untranslated region and intron.
- Intervening sequence; Sequence used for DNA cloning.
- *aad-12*; Modified version of an aryloxyalkanoate dioxygenase gene from *D. acidovorans*.

- Intervening sequence; Sequence used for DNA cloning.
- *AtuORF23*; 3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of *A. tumefaciens* pTi15955.
- Intervening sequence; Sequence from plasmid pENTR/D-TOPO and multiple cloning sites.
- CsVMV; Promoter and 5' untranslated region derived from the *Cassava vein mosaic virus*.
- Intervening sequence; Sequence used for DNA cloning.
- *pat*; Modified version of the phosphinothricin N-acetyl transferase (PAT) gene, isolated from *S. viridochromogenes*.
- Intervening sequence; Sequence from plasmid pCR12.1 containing multiple cloning sites.
- *AtuORF1*; 3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 of *A. tumefaciens* pTi15955.
- Intervening sequence; Sequence from Ti plasmid C58.
- T-DNA border A; Transferring DNA sequences.
- Intervening sequence; Sequence from Ti plasmid C58.
- T-DNA border A; Transferring DNA sequences.
- Intervening sequence; Sequence from Ti plasmid pTi15955.
- T-DNA border A; Transferring DNA sequences.

#### *Expressed proteins*

DAS-68416-4 soybean expresses the aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme originating from the soil bacterium *D. acidovorans*. AAD-12 is capable of deactivating several aryloxyalkanoate based herbicides, including phenoxy auxin (e.g., 2,4-D, MCPA) and pyridyloxy auxins (e.g., fluroxypyr, triclopyr). The *aad-12* gene was modified to allow optimal expression in plants. According to the applicant the wild type and plant optimised DNA sequences of *aad-12* are 79.7% identical.

DAS-68416-4 also expresses the phosphinothricin acetyltransferase (PAT) protein from *S. viridochromogenes*. 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.<sup>9</sup> As a result DAS-68416-4 is tolerant to glufosinate-ammonium based herbicides. The *pat* gene was also used as a selectable marker in the process of transgenic soybean regeneration.

#### *Molecular analysis*

The applicant confirmed by Southern blot analyses that a single copy of the *aad-12* and *pat* expression cassettes is integrated at a single integration locus in the genome of DAS-68416-4. Additionally, Southern blot analyses showed that the backbone sequences of plasmid pDAB4468 are absent in DAS-68416-4.

Sequence analysis of the DAS-68416-4 insertion site and its flanking sequences demonstrated that the flanking regions consist of soybean DNA and that the complete T-DNA sequence is present. The T-DNA insertion site was compared to the non-transgenic soybean genome. The

analysis revealed a 55 bp deletion from the original locus and a 9 bp insertion of unknown origin at the 3' integration junction of event DAS-68416-4.

The junctions between the insert and its flanking borders were identified and screened for novel open reading frames (ORFs). A total of twelve open reading frames (ORFs) spanning the junctions across the insert and its border regions in DAS-68416-4 were identified. Bio-informatic analysis from stop to stop codon using the allergen database (FARRP Allergen Online database, Version 10, January 2010) and GenBank non-redundant protein sequences did not indicate any sequence similarities between known toxins or allergens harmful to humans or animals and the putative polypeptides.

In view of the above, COGEM is of the opinion that the molecular characterisation of DAS-68416-4 has been adequately performed and meets the criteria laid down by COGEM.<sup>10</sup>

### **Environmental risk assessment**

The current application concerns import and processing of soybean line DAS-68416-4. In case of spillage soybean seed may be released into the environment. Soybean seeds rarely display dormancy, poorly survive in soil and do not survive freezing winter conditions. The Dutch climatic conditions are not optimal for growth of soybean. In the summer days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers.<sup>4</sup> In addition, volunteers are easily controlled mechanically or chemically.<sup>4</sup> Field trials with DAS-68416-4 soybean focusing on agronomic and phenotypic characteristics did not give any indication of increased weediness due to the presence of the AAD-12 and PAT proteins.

In view of the above, COGEM is of the opinion that the environmental risk of spread of soybean DAS-68416-4 within the Netherlands due to incidental spillage is negligible.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organisations.<sup>11</sup> This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations who advise 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. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

### **General surveillance plan**

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

The GS plan in this application states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorisation holder and operators involved in the handling and use of viable DAS-68416-4 soybean. In 2010, COGEM formulated criteria for GS plans concerning applications for import and cultivation of GM crops.<sup>12</sup> Although the GS plan

could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the GS plan sufficient for import and processing of DAS-68416-4 soybean.

### **Advice**

COGEM has been asked to advise on import and processing for use in food and feed of herbicide tolerant soybean line DAS-68416-4. This genetically modified soybean line expresses the *aad-12* gene and the *pat* gene conferring tolerance to several aryloxyalkanoate based and glufosinate-ammonium based herbicides. The molecular characterization of soy line DAS-68416-4 meets the criteria of COGEM.

Although field trials have indicated that some soybean varieties can be cultivated in the Netherlands, the Dutch climate is not optimal for soybean growth.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers. Modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds. There is no reason to assume that expression of the introduced *aad-12* and *pat* genes will increase the potential of soybean to establish feral populations. In addition, establishment of feral soybean populations in Europe has never been observed.

COGEM is of the opinion that the risk of spread of soybean DAS-68416-4 within the Netherlands due to incidental spillage of this soybean is negligible. Wild relatives of soybean are not present in Europe and therefore introgression of the inserted gene into closely related species can not occur.

Although the general surveillance (GS) plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current GS plan sufficient for import and processing of soybean line DAS-68416-4.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean DAS-68416-4 poses a negligible risk to the environment. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

### **Literature**

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