

# Advisory report concerning import of herbicide tolerant soybean SYHT0H2

## COGEM advisory report CGM/130325-01

### Summary

*The present application (EFSA/GMO/DE/2012/111) concerns import and processing for use in feed and food of the genetically modified soybean line SYHT0H2. This soybean line expresses the avhppd-03 gene and two pat genes. As a result, soybean SYHT0H2 is tolerant to HPPD inhibiting herbicides and to glufosinate ammonium containing herbicides.*

*The applicant showed by Southern blot analyses that two inverted and truncated copies of the insert are integrated at a single integration locus in the genome of soybean SYHT0H2 and that the backbone of the plasmid used for transformation is absent. Bioinformatic analysis of the junctions of the insert and the soybean genomic DNA identified eleven open reading frames (ORFs). These junction sequences were analysed for similarities to known toxins or allergens. No similarities were found. The applicant also analysed translations of all six reading frames of the T-DNA insert for similarities to known toxins or allergens. Although the applicant provides data on a single match of nine identical amino acids, the full set of blast results is missing.*

*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 such as seed shattering, dormancy and cold resistance. Establishment of feral soybean populations has never been observed in Europe. In addition, soybean volunteers are rarely observed throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers. COGEM considers the General Surveillance plan adequate for import of SYHT0H2.*

*COGEM is of the opinion that incidental spillage of SYHT0H2 poses a negligible risk to the environment. COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.*

*COGEM notes that the full set of blast results is missing. COGEM is of the opinion that these results should be available to risk assessors such as COGEM. Provided that the blast data is submitted and endorses the conclusions of the applicant, COGEM is of the opinion that the environmental associated with import and processing of soybean line SYHT0H2 are negligible.*

### Introduction

The present application by Syngenta Crop Protection AG (EFSA/GMO/DE/2012/111) concerns import, food, feed and processing of genetically modified (GM) soybean SYHT0H2. This soybean was produced by *Rhizobium radiobacter* (previously known as *Agrobacterium tumefaciens*<sup>1</sup>) mediated transformation of immature soybean seed and expresses the avhppd-03 gene derived from oat (*Avena sativa*) and two pat genes derived from *Streptomyces viridochromogenes*. As a result, soybean SYHT0H2 is tolerant to HPPD inhibiting herbicides, such as mesotrione, and to glufosinate ammonium containing herbicides.

### **Previous COGEM advice**

SYHT0H2 has not been previously assessed by COGEM. Nor has the COGEM advised on crops that are tolerant to HPPD inhibiting herbicides before. However, in the past COGEM has advised positively on the import and processing of several *pat* containing soybean lines such as DAS-68416-4, A2704-12 and A5547-127.<sup>2,3,4</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 favourable conditions a seedling will emerge in a 5-7 day period. Soybean is sensitive to frost and therefore does not survive freezing conditions.<sup>5,6</sup>

In the Netherlands, frost is common. On average 58 days in a year have a minimum temperature below 0°C.<sup>7,8</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 under temperate climatic conditions is possible.<sup>9,10</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>5</sup> As for all domesticated crops, one of the efforts of plant breeders was to reduce seed shattering in order to increase yield during harvesting. Soybean seeds rarely display dormancy and poorly survive in soil.<sup>11</sup> Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers.<sup>5</sup> In addition, volunteers are easily controlled mechanically or chemically.<sup>5</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>5</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>5</sup>

### **Molecular characterization**

Soybean SYHT0H2 was produced by transformation of immature soybean seed of conventional variety 'Jack' using the disarmed *R. radiobacter* strain EHA101 that contains the binary plasmid pSYN15954. An overview of the T-DNA region of the binary plasmid is given below:

- Right border region.
- FMV enhancer; *Figwort mosaic virus* (FMV) enhancer region which increases gene expression.
- 35S enhancer; *Cauliflower mosaic virus* (CaMV) 35S enhancer region.

- SMP promoter; synthetic minimal plant (SMP) promoter, derived from the *Cestrum yellow leaf curling virus* (CmYLCV).
- TMV enhancer; the 5' non-coding leader sequence from *Tobacco mosaic virus* (TMV), which functions as a translational enhancer in plants.
- *avhppd-03*; the gene *avhppd-03* derived from oat (*A. sativa*) which encodes the AvHPPD-03 enzyme that catalyzes the formation of homogentisic acid, the aromatic precursor of plastoquinone and tocopherol biosynthesis.
- NOS terminator; terminator sequence from the nopaline synthase (*nos*) gene of *R. radiobacter*.
- 35S promoter; promoter region of CaMV.
- *pat-03-01*; *S. viridochromogenes* strain Tü494 *pat* gene encoding the selectable marker PAT.
- NOS terminator; terminator sequence from the *nos* gene from *R. radiobacter*.
- CMP promoter; promoter and leader sequence from CmYLCV.
- TMV enhancer; the 5' non-coding leader sequence from TMV which functions as a translational enhancer in plants.
- *pat-03-02*; *S. viridochromogenes* strain Tü494 *pat* gene encoding the selectable marker PAT. PAT confers resistance to herbicides containing glufosinate ammonium (phosphinothricin).
- NOS terminator; terminator sequence from the *nos* gene from *R. radiobacter*.
- Left border region.

### ***Properties of the introduced genes***

SYHT0H2 expresses the *avhppd-03* gene encoding a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme (AvHPPD-03). HPPD enzymes catalyze the conversion of *p*-hydroxyphenylpyruvate (HPP) to homogentisic acid, which is an essential precursor in the biosynthesis of plastoquinone and tocopherols (more commonly known as vitamin E).<sup>12</sup> Mesotrione herbicides interfere with normal plant metabolism by tightly inhibiting plant HPPD. Inhibition of HPPD results in depletion of carotenoids. Under high light intensity, this leads to the destruction of chlorophyll molecules because the carotenoid pigments are no longer able to quench excess energy. This results in foliage bleaching, followed by necrosis and death.<sup>13</sup> The AvHPPD-03 isozyme that is produced in SYHT0H2 soybean has a lower binding affinity for HPPD-inhibiting herbicides than the native soybean HPPD. The production of the AvHPPD-03 isozyme thus confers tolerance to HPPD-inhibiting herbicides such as mesotrione.

In addition, SYHT0H2 expresses the *pat-03-01* and *pat-03-02* genes encoding phosphinothricin acetyltransferase enzymes (PAT). PAT confers tolerance to glufosinate-ammonium containing herbicides. The active ingredient in glufosinate-ammonium is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants.<sup>14</sup> L-PPT thereby inactivates glutamine synthetase, which leads to the accumulation of phytotoxic levels of ammonia in the plant. The PAT enzyme catalyses the conversion of L-PPT to an inactive form, which does not inhibit

glutamine synthetase.<sup>15</sup> Therefore, the application of glufosinate-ammonium containing herbicides to soybean SYHT0H2 will not result in the build-up of ammonia and subsequent plant death.<sup>16</sup>

### **Molecular analysis**

The applicant demonstrated by Southern blot, PCR and sequence analysis that soybean SYHT0H2 contains two inverted and truncated copies of the T-DNA at a single integration locus. The insert consists of a single copy of the *avhppd-03* gene, four copies of *pat* genes, a single copy of the *avhppd-03* enhancer complex sequence, two copies of the 35S promoter, two copies of the CMP promoter, two copies of the TMV enhancer (contained in the *pat-03-02* cassette) and five copies of the NOS terminator. In addition a 44 base pair DNA sequence that has similarity to the *avhppd-03* gene is located between the two copies of the insert. Also, there is a 17 base pair DNA insertion located in the 35S promoter of the 3' copy. The applicant demonstrated that SYHT0H2 does not contain backbone sequences of plasmid pSYN15954.

The applicant sequenced 1,000 base pairs of the 5' and 3' flanking regions. The results demonstrated that 15 bp from the soybean genomic sequence were deleted at the 3' insert-to-flank junction and 7 bp were inserted. Bioinformatic analysis (BLASTn, BLASTx) of these regions showed that both flanks are soybean genomic DNA and disruption of endogenous genes did not occur.

Bioinformatic analysis of the junctions of the insert and the soybean genomic DNA identified eleven open reading frames (ORFs) that are contained between stop codons. These sequences were analysed by alignment searches in an allergen database (FARRP Allergen Online database, 2012). An updated version of the NCBI Entrez® Protein Database was used as the source of known or putative toxin sequences. The results of these analyses demonstrated no sequence similarities between any known toxins or allergens and the eleven putative polypeptides.

The applicant states that translations of all six reading frames of the T-DNA insert of SYHT0H2 were evaluated for their similarity to allergens and toxins using BLASTX analysis. The applicant only provides data on a single match of nine identical amino acids between an alternate (putative) reading frame of the SYHT0H2 insert and three putative allergens from wheat. According to the applicant this hit is not biologically relevant since the sequence is located in an antisense orientation compared to the *avhppd-03* gene and is therefore not in the correct orientation to be expressed by the SMP promoter upstream. Furthermore, the applicant states that the same sequence is found in more than 16,000 proteins, which indicates it is not unique to known allergens.

Although there are no reasons to assume that the conclusions of the applicant are invalid, COGEM is of the opinion that the full set of blast results should be provided to substantiate

these conclusions. This is also important in the context of transparency and completeness of the data.

### **Environmental risk assessment**

The current application concerns import and processing of soybean line SYHT0H2. 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. Due to the characteristics of soybean, COGEM is of the opinion that the development and improvement of soybean varieties more suited for commercial cultivation in the Netherlands does not affect the environmental risk assessment of SYHT0H2 at this time.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers.<sup>5</sup> In addition, volunteers are easily controlled mechanically or chemically.<sup>5</sup>

There is no reason to assume that soybean line SYHT0H2 has an increased potential to survive or establish feral populations in case of incidental spillage.

COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.<sup>17</sup> This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. 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**

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

The current GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorization holder and operators involved in the handling and use of viable soybean.

In 2010, COGEM published a report on the principles that should be followed for general surveillance.<sup>18</sup> COGEM concluded that the GS plans could be improved by a guarantee that operators will monitor for unanticipated effects. In the present GS plan, the authorization holder states that the operators have agreed to provide information relevant to the monitoring of SYHT0H2 to the authorization holder. More important, it is stated that the authorization holder will be able to give evidence that the operators collect this information. This is in line with the criteria laid down by COGEM.<sup>18</sup> COGEM points out that a commitment of the applicant to

make raw monitoring data and the analyses thereof available to the Competent Authorities and the European Commission, could further improve the GS plan.<sup>19</sup> This request is also made by EFSA in the guidance document on post-market environmental monitoring.<sup>20</sup>

The GS plan states that if the authorisation holder identifies an unexpected adverse effect caused by the GM plant, he will inform the European Commission immediately. COGEM is of the opinion that Member States should also be directly informed of these effects by the authorisation holder, to ensure that appropriate measures for protection of humans and the environment can be implemented immediately.

### **Advice**

COGEM has been asked to advice on import and processing for use in food and feed of soybean line SYHT0H2. This line expresses the *avhppd-03* gene derived from oat (*A. sativa*) and the *pat-03-01* and *pat-03-02* genes derived from *S. viridochromogenes*. As a result, soybean SYHT0H2 is tolerant to HPPD inhibiting herbicides, such as mesotrione, and to glufosinate ammonium containing herbicides.

Although field trials have indicated that it might be possible to develop soybean varieties for cultivation 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 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 SYHT0H2 within the Netherlands due to incidental spillage of this soybean line is negligible. Wild relatives of soybean are not present in Europe and therefore introgression of the inserted transgenes into closely related species cannot occur. COGEM considers the current GS plan sufficient for import and processing of soybean line SYHT0H2. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Based on the information provided by the applicant, COGEM notes that the full set of blast results is missing. COGEM is of the opinion that these results should be made available to risk assessors such as COGEM. Provided that the blast data is submitted and endorses the conclusions of the applicant, COGEM is of the opinion that the environmental risks associated with import and processing of soybean line SYHT0H2 are negligible.

### **References**

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