Environmental risk assessment of import and processing of GM soybean GMB151 x DAS-44406-6

COGEM advice CGM/241127-01

- The present application (GMFF-2024-21774) concerns the authorisation of genetically modified (GM) soybean soybean GMB151 x DAS-44406-6 for import and processing for use in feed and food;
- The stacked event soybean GMB151 x DAS-44406-6 was produced by conventional crossbreeding of the two GM parental soybean lines;
- Previously, COGEM advised positively on the import and processing of both parental lines;

- GMB151 x DAS-44406-6 expresses the *2m epsps*, *aad-12* and *pat* genes conferring tolerance to glyphosate, 2,4-D and glufosinate-ammonium containing herbicides, respectively (from DAS-44406-6). It also contains the *hppdPf-4Pa* and *cry14Ab-1.b* genes (From GMB151) conferring tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-containing herbicides and resistance to plant pathogenic nematodes, respectively.

- In the Netherlands, feral soybean populations do not occur;
- Hybridisation of soybean with other species is not possible in the Netherlands;

- The molecular characterisation of GMB151 x DAS-44406-6 meets the criteria of COGEM;
- The bioinformatic analysis did not provide indications of potential environmental risks;

- There are no indications that the introduced traits allow the stacked event soybean GMB151 x DAS-44406-6 to survive in the Netherlands;
- There are no indications that GMB151 x DAS-44406-6 could establish feral populations in the Netherlands;

- COGEM is of the opinion that import and processing of soybean GMB151 x DAS-44406-6 poses a negligible risk to the environment in the Netherlands;
- COGEM abstains from giving advice on the potential risks of incidental consumption, as other organisations carry out a food/feed assessment.

1. Introduction

The present application (GMFF-2024-21774) filed by BASF Agricultural Solutions Seed LCC concerns import and processing of GMB151 x DAS-44406-6 (BCS-GM151-6 x DAS-444Ø6-6) soybean. The GM soybean was produced by conventional crossbreeding of the two genetically modified (GM) parental soybean lines. It expresses the, *hppdPf-4Pa*, *pat*, *aad-12* and *2mepsps* genes conferring tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, glufosinate-ammonium, 2,4-D

and glyphosate containing herbicides, respectively. Furthermore, it expresses the *cry14Ab-1.b* gene conferring resistance to nematodes, including the soybean cyst nematode.[1,2]

The parental lines GMB151[3] and DAS-44406-6[4] have been authorised for import and processing for use in food and feed in the European Union.

2. Previous COGEM Advice

Previously COGEM advised positively on import and processing of the parental lines GMB151[1] and DAS-44406-6.[2,5] COGEM also advised positively on the import and processing of a stacked event containing parental line DAS-44406-6; DAS-81419-2xDAS-44406-6.[6]

3. Environmental risk assessment

The objective of an environmental risk assessment (ERA) is to identify and evaluate potential adverse effects of the genetically modified organism (GMO), direct or indirect, immediate or delayed, on human health and the environment. This ERA involves the import and processing of GM soybean. Any concerns relating to cultivation, management or harvesting practices are beyond the scope of this advice. When assessing the environmental risk of incidental spillage of GM soybean COGEM first considers the likelihood that the event could establish itself in the Netherlands or could hybridise with related species. Other so-called 'areas of concern' (e.g. effects on non-target organisms) are addressed only if there is a chance that the event could establish itself or if gene flow to other species might occur.

3.1 Characteristics of the crop

Soybean (*Glycine max*) belongs to the Leguminosae (*Fabaceae*) family and is cultivated from equatorial to temperate zones. The optimum temperature for soybean growth is between 25 °C and 30 °C. Soybean is sensitive to frost and therefore does not survive freezing conditions.[7,8,9]

The soybean plant is not weedy in character.[8,9] To reduce yield losses during harvest, soybean plants with minimal seed scattering were selected for breeding. Soybean seeds rarely display dormancy, poorly survive in soil, and do not form a persistent soil seed bank.[8,10] Soybean volunteers are rarely observed throughout the world and do not compete effectively with other cultivated plants or primary colonisers.[8,9] In addition, volunteers are easily controlled mechanically or chemically.[9]

Soybean is a predominantly self-pollinating species. The anthers mature in the bud and directly pollinate the stigma of the same flower.[8,9] Pollinators such as honey bees (*Apis mellifera* L.) may improve the distribution of pollen on the stigmatic surface, which is known to increase seed set in many crops, and may as well facilitate transfer of soybean pollen and enable cross-pollination.[11,12,13,14] The cross-pollination rate of soybean is low and on average between 1 to 3%.[8,9,15,16,17,18,19] Usually, soybean pollen only disperse over short distances.

3.2 Receiving environment

As mentioned previously, soybean is sensitive to frost. Frost is common in the Netherlands, with an average of 51 days a year of minimum temperatures below 0 °C.[20] Although the Dutch climate is not optimal, soybean is cultivated on a small scale (6.4 hectares or approximately 1.6 acres in 2024).[21]

Soybean volunteers are very uncommon in the Netherlands and have never resulted in establishment of wild populations.[22,23] To the best of COGEM's knowledge, there are no reports of feral soybean populations in Europe. Additionally, hybridisation with other species is not possible in Europe because there are no wild relatives of soybean.[8,9]

Conclusion: In the Netherlands feral soybean populations do not occur and hybridization of soybean with other species is not possible.

3.3 Description of the introduced genes and traits

Soybean GMB151 x DAS-44406-6 soybean was created by conventional cross-breeding of the parental lines. For a description of the parental lines, see previous COGEM advices.[1,2] A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promotors and terminators).

Introduced genes	Encoded proteins	Regulatory elements	Traits
pat	Codon optimized variant of phosphininothricin N-acetyltransferase (PAT) originating from *Streptomyces viridochromogenes*[24]	CsVMV promoter from the cassava vein mosaic virus and AtuORF1 terminator from *Agrobacterium tumefaciens*	Tolerance to glufosinate-ammonium containing herbicides
2mepsps	The double mutant 5-enolpyruvylshikimate-3-phosphate synthase (2mEPSPS) enzyme originating from *Zea mays*[25]	Histone H4A748 promoter from *Arabidopsis thaliana* including an intron from the histone 3 gene from *A. thaliana* and Histone H4A748 terminator from *A. thaliana*	Tolerance to glyphosate containing herbicides
aad-12	Codon optimized version of aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme originating from *Delftia acidovorans*[26]	AtUbi10 constitutive promoter from the *A. thaliana* polyubiquitin 10 (UBQ10) gene and AtuORF23 terminator from *A. tumefaciens*	Tolerance to 2,4D containing herbicides
hppdPf-4Pa	Modified 4-hydroxy-phenylpyruvate dioxygenase (HPPD-4) from *Pseudomonas fluorescens*[27]	Double enhanced promoter from cauliflower mosaic virus (CaMV) and 35S terminator from CaMV	Tolerance to hydroxy-phenylpyruvate dioxygenase inhibitor containing herbicides
cry14Ab-1.b	Cry14Ab-1 from *Bacillus thuringiensis*[28]	AtUbi10 promoter from *A. thaliana* and 35S terminator from CaMV	Resistance to plant pathogenic nematodes

3.4 Molecular characterisation

Previously, COGEM evaluated the molecular characterisation of each parental line and considered these to be adequate.[1,2,5]

The applicant validated the presence of GMB151 x DAS-44406-6 by PCR and sequencing and compared the inserts and flanking sequences of GMB151 x DAS-44406-6 with the sequences of the corresponding single events GMB151 and DAS-44406-6. According to the applicant the inserts and flanking DNA regions in the stacked event are identical to the sequences determined previously for the respective single events. Furthermore, according to the applicant, expression of the inserted genes is similar to that of parent lines. The applicant performed bioinformatics analyses of the inserts and of the 5' and 3' flanking regions in GMB151 x DAS-44406-6 using databases updated in 2023. According to the applicant, the putative products of the open reading frames spanning the 5' and 3' junctions of the inserts did not generate any protein sequence similarity with known toxins, or other biologically active proteins.

COGEM is of the opinion that the molecular characterisation of GMB151 x DAS-44406-6 has been performed correctly and meets the requirements of COGEM.[29]

Conclusion: The molecular characterisation of soybean GMB151 x DAS-44406-6 is adequate and no indications of potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

Previously, COGEM evaluated the phenotypic and agronomic characteristics of each parental line of GMB151 x DAS-44406-6 and found no deviations influencing the outcome of the environmental risk assessment.[1,2] The applicant performed trials with GMB151 x DAS-44406-06 soybean for one growing season in 2022 at five sites in the USA. They were compared to its conventional counterpart U15-60620, and seven reference varieties (non-GMO). The applicant analysed the phenotypic and agronomic characteristics of GMB151 x DAS-44406-6 and found that most parameters were equivalent to non-GM soybean reference varieties, taking into account natural variation.

The only trait for which no equivalence between GMB151 x DAS-44406-06 and the non-GM references could be established was 'crop development' which was significant lower for GMB151 x DAS-44406-06 when treated with intended herbicides (TIH). However, the mean was within the range of the reference varieties and this difference was not seen with conventional herbicide management (CHM).

The results of the phenotypic evaluation do not give reason to assume that the GM soybean could pose an environmental risk. Therefore, COGEM is of the opinion that there are no indications that the introduced traits allow soybean GMB151 x DAS-44406-6 to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow GMB151 x DAS-44406-6 to survive in the Netherlands. GMB151 x DAS-44406-6 has no increased potential for the establishment of feral populations in the Netherlands.

4. Food/feed assessment

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 Wageningen Food Safety Research (WFSR). The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring

The applicant supplied a general surveillance plan as part of the PMEM. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan,[30,31] which is part of the PMEM plan, but considers the current GS (and PMEM) plan adequate for import and processing of GMB151 x DAS-44406-6 soybean.

Conclusion: The current PMEM plan is sufficient for the import and processing of GM soybean GMB151 x DAS-44406-6.

6. Overall conclusion

COGEM is of the opinion that import and processing of GMB151 x DAS-44406-6 soybean pose a negligible risk to the environment in the Netherlands. COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

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