Environmental risk assessment of import and processing of GM soybean DBN8002

COGEM advice CGM/231201-02

- The present application (GMFF-2022-11530) concerns the authorisation for import and processing for use in food and feed of genetically modified (GM) soybean DBN8002;
- The GM soybean expresses the *pat* gene conferring tolerance to glufosinate-ammonium containing herbicide and expresses the *vip3Aa* gene conferring resistance to certain plague insects;
- The molecular characterisation of DBN8002 meets the criteria of COGEM;
- In the Netherlands, feral soybean populations do not occur;
- Hybridisation of soybean with other species is not possible in the Netherlands;
- There are no indications that the introduced traits allow GM soybean DBN8002 to survive in the Dutch environment;
- COGEM is of the opinion that import and processing of GM soybean DBN8002 poses a negligible risk to the environment in the Netherlands;
- COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (GMFF-2022-11530), filed by Beijing DaBeiNong Biotechnology Co. Ltd, concerns the import and processing of genetically modified (GM) soybean DBN8002. DBN8002 expresses the *pat* gene conferring tolerance to glufosinate-ammonium containing herbicides. DBN8002 also expresses the *vip3Aa* gene conferring resistance to certain plague insects.

2. Previous COGEM advice

In the past, COGEM issued positive opinions on GM soybean events that express the *pat* gene (e.g. MON94313[1], DBN9004[2], A2704-12[3,4,5] and A5547-127[6,7]) conferring tolerance to glufosinate-ammonium containing herbicides. COGEM did not previously assess GM soybean expressing a *vip3Aa* gene, but did issue positive opinions on GM maize event MIR162 expressing *vip3Aa20* and GM cotton event COT102 expressing *vip3Aa19*.[8,9] Both genes are variants of a native *vip3Aa* gene, and differ by one amino acid from one another.

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 soybean

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.[10,11,12]

The soybean plant is not weedy in character.[11,12] 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.[11,13] Soybean volunteers are rarely observed throughout the world and do not compete effectively with other cultivated plants or primary colonisers.[11,12] In addition, volunteers are easily controlled mechanically or chemically.[12]

Soybean is a predominantly self-pollinating species. The anthers mature in the bud and directly pollinate the stigma of the same flower.[11,12] The cross-pollination rate of soybean is low and on average between 1 to 3%.[11,12,14,15,16,17,18] Soybean pollen disperses almost only 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.[19] Although the Dutch climate is not optimal, soybean is cultivated on a small scale (185 hectares or approximately 457 acres in 2022 according to provisional data).[20] Soybean volunteers are very uncommon in the Netherlands and have never resulted in establishment of wild populations.[21,22,23] To the best of COGEM's knowledge, there are no reports of feral soybean populations in Europe. Additionally, in Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[11,12]

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

3.3 Description of the introduced genes and traits

The GM soybean DBN8002 expresses the *pat* and *vip3Aa* genes. The *pat* gene encodes a phosphinothricin acetyltransferase (PAT) and is derived from the bacterium *Streptomyces viridochromogenes*.[24] The PAT enzyme confers tolerance to L-phosphinothricin, the active

ingredient of glufosinate-ammonium herbicides, by catalysing the acetylation of L-phosphinothricin to yield a harmless variant. The *vip3Aa* gene encodes an insecticidal protein. The vegetative insecticidal proteins (Vips) are originally identified in the vegetative growth phase of the bacterium *Bacillus thuringiensis*.[25] Vip3Aa is an intestine-specific virulence factor and shows insecticidal activity against lepidopteran species, such as *Agrotis ipsilon* (black cutworm), *Spodoptera frugiperda* (fall armyworm), *Spodoptera exigua* (beet armyworm), *Heliothis virescens* (tabacco budworm) and *Helicoverpa zea* (corn earworm).[25,26,27] The Vip3Aa variant produced in DBN8002 differs from the native Vip3Aa protein by one amino acid at position 284 (K to Q).

DBN8002 was generated by *Agrobacterium tumefaciens* (strain EHA101) mediated transformation with the pDBN4006 plasmid. This plasmid is approximately 11.3 kb and contains one T-DNA region (6200 bp), consisting of a *vip3Aa* expression cassette and a *pat* expression cassette. Outside the T-DNA, pDBN4006 contains the *aadA* gene from *Escherichia coli*. The *aadA* gene encodes an enzyme conferring tolerance to spectinomycin and streptomycin, and is used as a selectable marker for the plasmid in bacteria. With this transformation method, the T-DNA should integrate into the genome: the pDBN4006 backbone with the *aadA* gene should not be incorporated into the genome of the transformant. Following transformation, the applicant used several screening, selection and breeding steps to obtain DBN8002.

The inserted genetic elements, and a description thereof, are listed in the table below. The information in the table is limited to information on the introduced genes, corresponding traits, and regulatory elements.

Introduced genes	**Encoded proteins**	**Regulatory elements**	**Traits**
vip3Aa	Variant of a native vegetative insecticidal protein (Vip) class A, subclass a, (Vip3Aa) originating from *B. thuringiensis* train AB88	Promoter sequence from *Arabidopsis thaliana* (At*ACT2*), terminator sequence from *A. tumefaciens* (Nos)	Resistance against certain lepidopteran insects
pat	phosphinothricin N–acetyltransferase (PAT), codon optimised coding sequence originally derived from *S. viridochromogenes*	Promoter and terminator sequence (35S) from the cauliflower mosaic virus (CaMV)	Tolerance to glufosinate-ammonium containing herbicides

3.4 Molecular characterisation

The applicant used a series of Southern blot experiments, restriction digest, PCR amplification, and sequencing, combined with bio-informatic analyses to characterise DBN8002. For the Southern blot experiments, the applicant used 12 DNA probes (between 546 and 1145 bp in size): 7 probes specific for the T-DNA insert and 5 probes covering the plasmid backbone. The Southern blot experiments

demonstrate the absence of the plasmid backbone sequence in DBN8002. It was demonstrated that DBN8002 contains a single intact insert, which remains stable across five generations.

The insertion and flanking sequences in the genomic DNA of DBN8002 were further analysed via PCR amplification experiments and subsequent sequencing. Analysis of the sequencing data shows the inserted T-DNA in DBN8002 to be identical to the T-DNA of the transformation plasmid pDBN4006. The applicant analysed the integrity of the site of insertion by comparing its sequence to the sequence of the genome of conventional soybean. Bio-informatic analysis of about 1 kb flanking either side of the insert in DBN8002, show the T-DNA to be inserted on chromosome 3. A 6 bp deletion was found at the T-DNA integration site. The applicant states that the deleted region corresponds to an intergenic region, and did not disrupt any known gene or annotated sequence of the soybean genome. According to analysis of the insertion site, no endogenous genes were disrupted by the T-DNA insert.

All six reading frames of the T-DNA insert and the junctions between the insert and the soybean genome were translated from stop-to-stop codon into putative amino acid sequences and evaluated for potential similarity to known toxins and allergens that could affect human or animal health. Multiple databases were consulted for the bioinformatic analyses, amongst which the COMPARE database, NCBI's RefSeq Protein Database and UniProtKB. The applicant used a FASTA sequence alignment tool to compare the putative amino acid sequences and allergen databases for similarities. In this analysis no matches to allergens were found. The applicant used BLASTP to compare the putative amino acid sequences against toxin databases for similarities. Two putative open reading frames (ORFs) were found to share similarity with known toxins. One ORF consists of the *Vip3Aa* gene and part of its promoter. The known toxins it was found to share similarity with was the insecticidal Vip3 protein itself. The other ORF consists of the *pat* gene and part of its promoter. This ORF shares similarity with GNAT family members that are usually composed of a toxin-antitoxin system. The toxin component is a protein with N-acetyltransferase activity. The applicant states that no records were found that identified potential hazards associated with this protein family. The bioinformatic analysis did not identify any other potential ORFs with sequence similarities to known toxic proteins. Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.[28]

Conclusion: The molecular characterisation of soybean DBN8002 is adequate and no indications for potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

The applicant observed thirteen phenotypic and agronomic characteristics of DBN8002 at eight field sites in China and compared them to the conventional counterpart ('Jack') and several conventional reference varieties. For 12 out of 13 characteristics, no significant difference between DBN8002 and the conventional control, and equivalence between DBN8002 and the reference varieties, was found. For one of the assessed characteristics ('1000 seeds weight'), the equivalence of DBN8002 and the reference varieties was observed to be 'more likely than not'. As no significant difference was found

between DBN8002 and the conventional control, the applicant did not conduct any further analysis. Treatment of DBN8002 with glufosinate-containing herbicides had no impact on the agronomic and phenotypic characteristics.

The applicant analysed the composition of soybean grain and focussed on 7 traits, amongst which carbohydrate and crude protein composition. For 2 out of 7 traits, ash and moisture, no significant difference was found but no conclusion could be drawn on the equivalence between DBN8002 and the reference varieties. The applicant states that the values were within the range of the reference varieties.

The germination of DBN8002 seeds was compared with the conventional counterpart and five reference varieties. Measurements of normal germination, abnormal germination, hard, or firm-swollen seeds were taken. All values fell within the range of the reference varieties, and no statistical differences were identified between DBN8002 and the conventional control.

The applicant also tested whether DBN8002 has an enhanced survivability or competitive advantage over conventional soybean. The plant height, compound leaf number, plant coverage, propagation, and seed shattering of the plants were assessed on both uncultivated and cultivated land. No significant differences were observed, indicating that DBN8002 has no competitive advantage over conventional soybean.

COGEM assessed the above mentioned results and concludes that – except for the introduced herbicide tolerance and insect resistance traits – the agronomic and phenotypic characteristics of DBN8002 are comparable to conventional soybean varieties. There are no indications that DBN8002 soybean will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that soybean DBN8002 would be able to survive or establish 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, WFSR carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a post-market environmental monitoring (PMEM) plan. The general surveillance (GS) plan differs in specific details, but is overall comparable to the GS plans from previous applications of GM crops. In this GS plan, the applicant has not specified on the communication and agreements with third parties, but consults the same associations as stated in comparable GS plans. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[29,30] but considers the current GS plan adequate for import and processing of soybean DBN8002.

Conclusion: The current PMEM plan of gg-soybean DBN8002 is sufficient for import and processing.

6. Overall conclusion

COGEM is of the opinion that import and processing of soybean DBN8002 poses 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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