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DATUM	03 juli 2024
KENMERK	CGM/240703-01
ONDERWERP	Advies import en verwerking van gg-soja MON94637

Geachte heer Jansen,

Naar aanleiding van een adviesvraag over de milieurisico's van import en verwerking van gg-soja MON94637 (GMFF-2023-21116; AP188), ontwikkeld door Bayer Agriculture, deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren over eventuele milieurisico's van import en verwerking van de genetisch gemodificeerde (gg-)sojalijn MON94637. Deze sojalijn zal niet als individuele lijn worden gecommercialiseerd, maar zal alleen gebruikt worden als kruisingsouder om nieuwe gg-sojavarianten mee te produceren die meerdere transgenen bevatten (stapelen). De beoordeling omhelst het hypothetische geval dat deze gg-soja geïmporteerd wordt. In de gg-sojalijn MON94637 komen de genen *cry1A.2* en *cry1B.2* tot expressie, waardoor de plant resistent is tegen bepaalde plaaginsecten die behoren tot de vlinderachtigen.

Hoewel het klimaat niet optimaal is, wordt soja op kleine schaal in Nederland geteeld. Opslagplanten worden hier zelden waargenomen en hebben nooit geleid tot verwilderde populaties. Er zijn geen wilde verwanten van soja aanwezig in Europa, waardoor de ingebrachte insectenresistentiegenen zich niet naar andere soorten kunnen verspreiden. De moleculaire karakterisatie van MON94637 voldoet aan de eisen van de COGEM. Er zijn geen redenen om aan te nemen dat expressie van de ingebrachte genen ervoor zorgt dat deze gg-sojalijn zich in Nederland zou kunnen vestigen.

Alle aspecten in overweging nemende, acht de COGEM de milieurisico's voor Nederland bij de (hypothetische) import en verwerking van gg-soja MON94637 verwaarloosbaar klein. Omdat andere instanties een voedselveiligheidsbeoordeling uitvoeren, heeft de COGEM bij deze vergunningaanvraag de risico's van incidentele consumptie niet beoordeeld.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ing. Sybe Schaap
Voorzitter COGEM

c.c.

- Drs. Y. de Keulenaar, Hoofd Bureau ggo
- Ministerie van IenW, Directie Omgevingsveiligheid en milieurisico's, DG Milieu en Internationaal
- Ing. M.A.C. Möllers, Food-Feed loket

Environmental risk assessment of import and processing of GM soybean MON94637

COGEM advice CGM/240703-01

- The present application (GMFF-2023-21116; AP188) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) soybean MON94637;
- GM soybean MON94637 will not be commercialised as a single product, the environmental risk assessment therefore considers the hypothetical case in which import could occur;

- The GM soybean expresses the *cry1A.2* and *cry1B.2* genes conferring resistance to certain lepidopteran insects;

- The molecular characterisation of soybean MON94637 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 MON94637 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of GM soybean MON94637 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.

1. Introduction

The present application (GMFF-2023-21116; AP188), filed by Bayer Agriculture, concerns the import and processing of genetically modified (GM) soybean MON94637. MON94637 expresses the *cry1A.2* and *cry1B.2* genes conferring resistance to certain lepidopteran insects. The applicant notes that this event will be used as a parental line to generate stacked events, but will not be commercialised as a single product.

2. Previous COGEM Advice

COGEM has not previously assessed GM soybean expressing the *cry1A.2* and *cry1B.2* genes but has advised positively on GM soybean events that express other *cry* genes conferring resistance to certain lepidopteran insects.[1,2,3,4,5]

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.[6,7,8]

The soybean plant is not weedy in character.[7,8] 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.[7,9] Soybean volunteers are rarely observed throughout the world and do not compete effectively with other cultivated plants or primary colonisers.[7,8] In addition, volunteers are easily controlled mechanically or chemically.[8]

Soybean is a predominantly self-pollinating species. The anthers mature in the bud and directly pollinate the stigma of the same flower.[7,8] 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.[10,11,12,13] The cross-pollination rate of soybean is low and on average between 1 to 3%.[7,8,14,15,16,17,18] 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.[19] Although the Dutch climate is not optimal, soybean is cultivated on a small scale (442 hectares or approximately 1092 acres in 2023).[20] Soybean volunteers are very uncommon in the Netherlands and have never resulted in establishment of wild populations.[21,22] 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.[7,8]

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

GM soybean MON94637 expresses the *cry1A.2* and *cry1B.2* genes conferring resistance to certain lepidopteran insects. The c*ry1A.2* gene encodes for a chimeric protein containing domains derived from wildtype cry1 proteins of the cry1A and cry1C subclasses. Chimeric cry1A.2 shows higher insecticidal activity against *Spodoptera eridania* (southern armyworms) compared to the wildtype protein and shows insecticidal activity against other lepidopteran species, such as *Spodoptera exempta* (black armyworm), *Chrysodeixis includens* (soybean looper) and *Anticarsia gemmatalis* (velvetbean caterpillar).[23] The *cry1B.2* gene encodes for a chimeric protein containing domains derived from wildtype cry1 proteins of the cry1A, cry1B and cry1K subclasses. This chimeric protein results in higher insecticidal activity against *Spodoptera frugiperda* (fall armyworms) compared to the wildtype protein. In addition, cry1B.2 shows insecticidal activity against lepidopteran species, such as *Spodoptera eridania* (southern armyworms), *Spodoptera exempta* (black armyworm), *Chrysodeixis includens* (soybean looper) and *Anticarsia gemmatalis* (velvetbean caterpillar) and *Helicoverpa armigera* (old-world bollworm).[23]

MON94637 was generated by *Agrobacterium tumefaciens* (strain A3555) mediated transformation with the PV-GMIR527237 plasmid. This plasmid contains two T-DNA regions (T-DNA I and II). The *cry1A.2* and *cry1B.2* expression cassettes conferring resistance to certain lepidopteran insects are located on T-DNA I. T-DNA-II contains the *aadA* and *splA* expression cassettes, which are used as selectable markers and confer tolerance to spectinomycin and streptomycin (*aadA*) or give a scorable phenotype, i.e. shrunken seeds(*splA*).[24] Following transformation, the applicant used several screening, selection, and segregation steps to obtain plants that contain a single copy of T-DNA-I and no T-DNA-II or plasmid backbone sequences.

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**
cry1A.2	Chimeric protein with domains from different cry1 proteins, cry1A and cry1C subclasses, from *Bacillus thuringiensis*[23]	Promoter, leader and intron of the polyubiquitin gene *ubq10* from *Arabidopsis thaliana* 3'UTR sequence of the *Zfp-Mt1* gene from *Medicago truncatula*	Resistance against certain lepidopteran insects
cry1B.2	Chimeric protein with domains from different cry1 proteins, cry1A and cry1B and cry1K subclasses, from *B. thuringiensis*[23]	Promoter and leader of a chlorophyll a/b-binding (CAB) protein from *Cucumis melo* 3' UTR sequence from the *Lox-Mt1* gene of *M. truncatula*	Resistance against certain lepidopteran insects

3.4 Molecular characterisation

The applicant used next-generation sequencing and directed sequencing combined with bioinformatic analyses to characterise MON94637. The obtained results indicate that MON94637 contains a single intact insert of the T-DNA-I, which remains stable across five generations. In certain MON94637 generations, low coverage reads of plasmid sequences were found, which are likely detected due to the presence of environmental bacteria and do not indicate the presence of backbone sequences, as stated by the applicant. The results demonstrate the absence of the plasmid backbone sequence in MON94637.

The insertion and flanking sequences in the genomic DNA of MON94637 were further analysed via PCR amplification experiments and subsequent sequencing. Analysis of the sequencing data shows the inserted T-DNA I, containing the *cry1A.2* and *cry1B.2* expression cassettes, in MON94637 to be identical to the T-DNA of the transformation plasmid V-GMIR527237. The 5’ and 3’ flanking regions (1 kb each) of the insert in MON94637 were sequenced as well. The applicant analysed the integrity of the site of insertion by comparing its sequence to the sequence of the corresponding region in the conventional control (soybean A3555). The results from this analysis show that 14 basepairs (base 1,001-1,014) were deleted, most likely upon insertion of the T-DNA-I. The applicant states that such changes are common during plant transformation and these changes presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process.[25] Bioinformatic analysis of the site of insertion indicates that the T-DNA-I was inserted at chromosome 19 of the soybean genome and that no endogenous genes were disrupted by the insertion. COGEM notes that the deletion of 14 basepairs upon T-DNA insertion is unlikely to result in potential environmental risks as no endogenous genes were disrupted.

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. The applicant used a FASTA sequence alignment tool to compare the putative amino acid sequences and allergen databases. Multiple databases were consulted for the bioinformatic analyses, amongst which the AD_2023, TOX_2023 and PRT_2023 databases. In addition to the ‘FASTA search’ an 8- and an 80-amino acid sliding window search was used to identify epitopes that are potentially allergenic. No sequence similarities with known putative allergens, toxins or other biologically active proteins that may be harmful for human or animal health, were detected using a so-called ‘FASTA search’ or the sliding window searches. Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.[26]

Conclusion: The molecular characterisation of GM-soybean MON94637 is adequate and no indications for potential environmental risks were identified.

3.5 Phenotypic and agronomic characteristics

The applicant observed ten agronomic and phenotypic characteristics of MON94637 at eight field sites in the US and compared them to the conventional counterpart and several conventional reference varieties. Some of the assessed characteristics MON94637 were observed to differ from the conventional counterpart. In herbicide-treated MON94637 soybean plants differences were observed with regard to ‘final stand count’ (number of plants per m^2, higher than control) and ‘yield’ (harvested grain (t/ha) adjusted to 13% moisture content, lower than control). The above mentioned characteristics were within the range of the reference varieties (‘equivalent’, or ‘equivalence more likely than not’). No differences between MON94637 and its conventional counterpart were observed in abiotic stress response, arthropod pest damage and disease damage.

The applicant also studied germination of MON94313 seeds. The germination of MON94637 seeds was compared with the conventional counterpart and four reference varieties. Measurements of percentage of germinated seeds, percentage of dead seeds, hard, or firm-swollen seeds were taken. No statistical differences were identified between MON94637 and the conventional control.

COGEM assessed the above mentioned results and concludes that – except for the introduced insect resistance traits – the agronomic and phenotypic characteristics of MON94637 are comparable to conventional soybean varieties. There are no indications that MON94637 soybean will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that soybean MON94637 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. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[27,28] but considers the current GS plan adequate for import and processing of soybean MON94637.

Conclusion: The current PMEM plan is sufficient for the (hypothetical) import and processing of GM soybean MON94637.

6. Overall conclusion

COGEM is of the opinion that import and processing of soybean MON94637 (a hypothetical case, as GM soybean MON94637 will not be commercialized as a standalone product) 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.

7. Additional remark regarding hypothetical import

COGEM notes that although an application for import and processing of MON94637 was filed, the applicant states that it will not be commercialised as a single event and will only be used to create stacked events. This situation results from the procedures followed by EFSA, i.e. that an application for import and processing of a stacked GM line can only be filed if the parental GM lines have been assessed.[29] COGEM points out that in this particular case it is not relevant to assess the single event MON94637, because it will never be commercialised as a standalone product. COGEM considers the request for authorisation of MON94637 for import and processing and use in food and feed as an example of following unnecessary procedures.

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