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KENMERK CGM/230210-01
ONDERWERP Advies import en verwerking van de herbicidentolerante sojalin MON94313

Geachte mevrouw Heijnen,


Naar aanleiding van een adviesvraag over de milieurisico's van import en verwerking van gg-soja MON94313 (EFSA/GMO/NL/2022/176), ontwikkeld door Bayer Agriculture BV, deelt de COGEM u het volgende mee.

Samenvatting:

De COGEM is gevraagd om te adviseren over eventuele milieurisico's van import en verwerking van de genetisch gemodificeerde (gg-) sojalin MON94313. Deze sojalin brengt de *dmo*, *pat*, *ft_t.1* en *tdo* genen tot expressie en is daardoor tolerant voor dicamba, glufosinate-ammonium, 2,4-D en mesotrione bevattende herbiciden. MON94313 zal niet als individuele lijn worden gecommercialiseerd, maar alleen gebruikt worden als kruisingsouder.

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 herbicidetolerantiegenen zich niet naar andere soorten kunnen verspreiden. De moleculaire karakterisering van gg-soja MON94313 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-sojalin zich in Nederland zou kunnen vestigen.

Alle aspecten in overweging nemende, acht de COGEM de milieurisico's voor Nederland bij import en verwerking van gg-soja MON94313 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
Vorzitter 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 herbicide tolerant soybean MON94313

COGEM advies CGM/230210-01

- The present application (EFSA/GMO/NL/2022/176) concerns the authorisation for import and processing for use in food and feed of genetically modified (GM) soybean MON94313;
- MON94313 will be used as a parental line to generate stacked events, but will not be commercialised as a single product;
- The GM soybean expresses the *dmo*, *pat*, *ft_t.1* and *tdo* genes conferring tolerance to dicamba, glufosinate-ammonium, 2,4-D and mesotrione containing herbicides;
- The molecular characterisation of MON94313 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 MON94313 to survive in the Dutch environment;
- COGEM is of the opinion that import and processing of GM soybean MON94313 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 (EFSA/GMO/NL/2022/176), filed by Bayer Agriculture BV on behalf of Bayer CropScience LP, concerns import and processing of genetically modified (GM) soybean MON94313. MON94313 expresses the *dmo*, *pat*, *ft_t.1* and *tdo* genes conferring tolerance to dicamba, glufosinate-ammonium, 2,4-D and mesotrione containing herbicides. 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

In the past, COGEM issued positive opinions on a GM soybean event that expresses a *dmo* gene (i.e. MON87708¹) resulting in tolerance to dicamba containing herbicides, and on GM soybean events that express the *pat* gene (e.g. A2704-12^{2,3,4} and A5547-127^{5,6}) conferring tolerance to glufosinate ammonium containing herbicides.

COGEM did not previously advise on GM soybean expressing *ft_t.1* genes, but did advise positively on a GM maize event (i.e. MON87429) that expresses a *ft_t* gene,⁷ which confers tolerance to 2,4-D containing herbicides. The *ft_t.1* gene which is introduced in MON94313 soybean was derived from this *ft_t* gene.⁸ COGEM also issued positive opinions on GM soybean events which express other transgenes that confer tolerance to 2,4-D.^{9,10}

COGEM did not previously assess GM crops expressing *tdo* genes, but did issue positive opinions on GM soybean events that express other transgenes conferring tolerance to mesotrione containing herbicides.^{11,12,13}

3. Environmental risk assessment

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.^{14,15,16} In the Netherlands frost is common. On average 51 days a year the minimum temperature is below 0°C.¹⁷ Although the Dutch climate is not optimal, soybean is cultivated on a small scale.

The soybean plant is not weedy in character.^{15,16} To reduce yield losses during harvesting, soybean plants that have 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.^{15,18} Soybean volunteers are rarely observed throughout the world and do not effectively compete with other cultivated plants or primary colonisers.^{15,16} In addition, volunteers are easily controlled mechanically or chemically.¹⁶ Soybean volunteers are very uncommon in the Netherlands and have never resulted in the establishment of wild populations.^{19,20} To the best of COGEM's knowledge, there are no reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species. The anthers mature in the bud and directly pollinate the stigma of the same flower.^{15,16} The cross-pollination rate of soybean is low and on average between 1 to 3%.^{15,16,21,22,23,24,25} Soybean pollen disperses only over short distances. In Europe, wild relatives of soybean do not occur. Hybridisation of soybean with other species is therefore not possible.^{15,16}

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

3.2 Description of the introduced genes and traits

MON94313 was generated by *Agrobacterium tumefaciens* mediated transformation with the PV-GMHT529103 plasmid. This plasmid contains two T-DNA regions (T-DNA I and II). The *dmo*, *pat*, *ft_t.1* and *TDO* expression cassettes conferring tolerance to several herbicides 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 seeds²⁶ (*spla*). 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 vector 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
<i>dmo</i>	dicamba mono-oxygenase (DMO), codon optimised coding sequence originally derived from <i>Stenotrophomonas maltophilia</i>	promoter, leader and intron of the polyubiquitin gene <i>ubq3</i> from <i>Arabidopsis thaliana</i> chloroplast targeting sequence of the <i>APG6</i> gene from <i>A. thaliana</i> 3'UTR sequence of the <i>Sali3-2</i> gene from <i>Medicago truncatula</i>	tolerance to dicamba containing herbicides
<i>pat</i>	phosphinothricin N-acetyltransferase (PAT), codon optimised coding sequence originally derived from <i>Streptomyces viridochromogenes</i>	synthetic promoter and 5'UTR based on multiple promoter and 5'UTR sequences from <i>A. thaliana</i> synthetic intron based on multiple intron sequences from <i>A. thaliana</i> 3'UTR sequence of a putative <i>Hsp20</i> gene from <i>M. truncatula</i>	tolerance to glufosinate-ammonium containing herbicides
<i>ft_t.1</i>	FOPs and 2,4-D dioxygenase (FT_T.1), a modified version of the R-2,4 dichlorophenoxypropionate	promoter, leader and intron of the polyubiquitin gene <i>ubq10</i> from <i>A. thaliana</i>	tolerance to members of the synthetic auxin herbicide family, such as 2,4-D

	dioxygenase (<i>rdpA</i>) gene of <i>Sphingobium herbicidovorans</i>	3' UTR sequence from an expressed gene of unknown function from <i>M. truncatula</i>	in maize FT_T.1 also confers tolerance to aryloxyphenoxypropionate (FOP) containing herbicides, but soybean is tolerant to FOP by nature
<i>tdo</i>	triketone dioxygenase (TDO), codon optimised coding sequence originally derived from <i>Oryza sativa</i>	synthetic promoter and 5'UTR based on multiple promoter and 5'UTR sequences from <i>A. thaliana</i> synthetic intron based on multiple intron sequences from <i>A. thaliana</i> synthetic 3'UTR based on multiple 3'UTR sequences from <i>Z. mays</i>	tolerance to β -Triketone herbicides, such as mesotrione

3.3 Molecular characterisation

The applicant used next-generation sequencing and directed sequencing combined with bioinformatic analyses to characterise MON94313. The obtained results indicate that MON94313 contains a single intact copy of the T-DNA-I. The border regions of the T-DNA-I are truncated. T-DNA-II and vector backbone sequences are not present in MON94313.

The insert in MON94313 was sequenced and consists of 10,196 basepairs. Bioinformatic analyses indicate that it is identical to the sequence of the corresponding region of the plasmid vector PV-GMHT529103. The 5' and 3' flanking regions (1 kb each) of the insert in MON94313 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 40 basepairs were deleted, most likely upon insertion of the T-DNA-I. Bioinformatic analysis of the site of insertion indicates that the T-DNA-I was inserted at chromosome 13 of the soybean genome and that no endogenous genes were disrupted by the insertion.

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 polypeptide sequences and evaluated for potential similarity to known toxins, allergens or biologically active proteins that could affect human or animal health. The databases that were used for the bioinformatic analyses, were assembled in 2021. 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'. In addition to the 'FASTA search' an eight amino acid sliding window search was used to identify epitopes that are potentially allergenic. In this analysis a match to a wheat allergen was found. The assessment of potential allergenicity is not part of the environmental risk assessment, but is included in the food/feed safety assessment which is carried out by EFSA and WFSR.

Overall, the molecular characterisation was conducted according to the criteria previously laid down by COGEM.²⁷

Conclusion: The molecular characterisation of soybean MON94313 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant studied germination of MON94313 seeds using an optimum and a suboptimum temperature regime. In both temperature regimes the percentage of germinated seeds of MON94313 was higher (and the percentage of dead seeds lower) than the conventional control, but within the range of the germination percentage of the reference varieties.

The applicant also observed ten phenotypic characteristics of MON94313 at eight field sites in the US and compared them to the conventional counterpart and several conventional reference varieties. For some of the assessed characteristics MON94313 was observed to differ from the conventional counterpart. In herbicide treated MON94313 soybean plants differences were observed with regard to 'plant height' and 'days to maturity', and in non-herbicide treated MON94313 plants 'days to flowering' differed. 'Seed weight' was different in both treatments. The above mentioned characteristics were within the range of the reference varieties ('equivalent', or 'equivalence more likely than not'). No differences between MON94313 and its conventional counterpart were observed in abiotic stress response, arthropod pest damage and disease damage.

COGEM assessed the above mentioned results and concludes that - except for the introduced herbicide tolerance traits - the agronomic and phenotypic characteristics of MON94313 are comparable to conventional soybean varieties. There are no indications that MON94313 soybean will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that MON94313 soybean 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^{28,29} but considers the current GS plan adequate for import and processing of soybean MON94313.

6. Overall conclusion

COGEM is of the opinion that import and processing of soybean MON94313 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

COGEM notes that although an application for import and processing of MON94313 was filed, the applicant states that it will not be commercialised as a single event and will only be used as a parental line 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.³⁰ COGEM is of the opinion that there is no need to file an application for a single event that will never be commercialised. Potential risks of the single events used to create a stacked event can be assessed during the risk assessment of the stacked event.

COGEM considers the assessment of single events which will not be commercialised as stand-alone products superfluous and considers the request for authorisation of MON94313 for import and processing and use in food and feed an example of following unnecessary procedures. These procedures result in unnecessary delays in the assessment of stacked GM events.

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