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KENMERK CGM/170929-01
ONDERWERP Advies import en verwerking gg-katoen COT102

Geachte mevrouw Dijkma,

Naar aanleiding van een adviesvraag over de milieurisico's van import en verwerking van de genetisch gemodificeerde katoenlijn COT102 (EFSA/GMO/DE/2017/141), ingediend door Syngenta, deelt de COGEM u het volgende mee.

Samenvatting:

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van de genetisch gemodificeerde (gg-)katoenlijn COT102. In deze lijn komen de genen *vip3Aa19* en *aph4* tot expressie, waardoor het gewas resistent is tegen vraat van bepaalde vlinderachtigen, en resistent is tegen het antibioticum hygromycine B.

Het Nederlandse klimaat is niet geschikt voor de teelt van katoen. Er komen ook geen wilde verwanten van katoen voor in Noordwest-Europa, waardoor de ingebrachte sequenties van katoenlijn COT102 zich niet naar andere soorten kunnen verspreiden.

De moleculaire karakterisering van katoenlijn COT102 voldoet aan de eisen van de COGEM. Er zijn geen redenen om aan te nemen dat de eigenschappen die in katoenlijn COT102 zijn ingebracht, ervoor kunnen zorgen dat deze gg-katoenlijn kan verwilderen in Nederland. Mede daarom acht de COGEM de kans verwaarloosbaar klein dat het incidenteel morsen van gg-katoenzaden leidt tot verspreiding of vestiging van COT102 in Nederland.

Alle aspecten in overweging nemende, acht de COGEM de milieurisico's voor Nederland bij import en verwerking van gg-katoen COT102 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,



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Import and processing of genetically modified cotton COT102

COGEM advice CGM/170929-01

- The present application (EFSA/GMO/DE/2017/141) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) cotton (*Gossypium hirsutum*) COT102;
- GM cotton COT102 expresses the *vip3Aa19* and *aph4* genes, which confer resistance against certain lepidopteran insects and against the antibiotic hygromycin B.

- In the Netherlands, cultivation of cotton is not possible and feral cotton populations do not occur;
- Wild relatives of cotton are not present in the Netherlands, so hybridisation with other species is not possible;

- The molecular characterisation of cotton COT102 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of cotton COT102;
- There is no reason to assume that the introduced traits will allow GM cotton COT102 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of cotton COT102 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/DE/2017/141), filed by Syngenta, concerns import and processing of genetically modified (GM) cotton (*Gossypium hirsutum*) COT102. This GM cotton line contains the *vip3Aa19* and *aph4* genes, conferring resistance against certain lepidopteran insects, and against the antibiotic hygromycin B.

2. Previous COGEM advice

COGEM has not previously advised on import and processing of GM cotton COT102. COGEM did advise positively on a crop containing a *vip3A* gene, GM maize MIR162. This maize line contains the gene *vip3Aa20*, which differs from *vip3Aa19* by one amino acid.¹ COGEM also has not yet advised on GM crops conferring hygromycin B resistance. In 2007, a generic advice was published on the use of antibiotic resistance genes, but hygromycin B was not mentioned.² The European Food Safety Authority (EFSA) published a scientific opinion on the use of antibiotic

resistance genes as marker genes in 2004.³ In this EFSA publication, hygromycin B was classified as a group I antibiotic resistance gene, indicating that it is extremely unlikely that the presence of this gene affects human and animal health, or will impact the already existing spread of antibiotic resistance genes in the environment.³

3. Environmental risk assessment

3.1 Aspects of the wild-type crop

Cotton is a member of the genus *Gossypium* and belongs to the *Malvaceae* family. The majority of cultivated cotton is *Gossypium hirsutum* (90%), followed by *Gossypium barbadense* (5%), and *Gossypium arboreum* and *Gossypium herbaceum* (together $\leq 5\%$).^{4,5,6} The only cultivated cotton species in Europe is *G. hirsutum*, which is grown in Greece, Spain and Bulgaria.^{7,8}

Cotton requires at least 500 mm of rainfall during the growing season, but can also be grown as irrigated crop.⁵ Cotton is highly sensitive to temperature, and susceptible to frost.^{5,6,9,10} Seed germination and plant development cease below a temperature of 12 °C and delay when the temperature rises above 38 °C.^{5,6} The optimal daytime temperature for *G. hirsutum* ranges between 30 and 35 °C.^{5,10} *G. hirsutum* requires 180 to 200 frost-free days of uniformly high temperatures (averaging 21-22 °C) after planting.^{6,11} From planting of cotton to 60% boll opening (i.e., when seed is mature), a minimum of 2050 day degrees* is required.^{6,12} In the Netherlands, there are on average 85 days with a daily maximum temperature of ≥ 20 °C per year.¹³ Frost days in the Netherlands generally occur from October up to and including April¹⁴, and it is not uncommon for frost days to occur in early summer. In the summer (June, July and August), when temperatures are highest, the daily temperature averages 17 °C.^{15,16} This corresponds to an accumulated average of 436 day degrees. In the remaining months, the temperature is insufficiently high to reach the accumulated amount of day degrees required (2050) for the growth and maturation of cotton. Considering the above, the Dutch climate conditions are unsuitable for the life cycle of cotton.

Cotton plants reproduce sexually.⁶ Cotton is predominantly a self-pollinating species, but cross-pollination may occur.¹⁷ Dissemination of pollen by wind is (almost) absent.^{5,6} Outcrossing rates for cotton are strongly influenced by the presence of insects. Cotton seeds can remain dormant for 2-3 months, but this trait is reduced or eliminated by selective breeding. Seeds from modern cotton cultivars do not possess dormancy.⁵ Cotton seeds from cultivars usually do not survive in humid soil and the formation of seed banks is unlikely, as seeds become weathered when they do not germinate directly.^{6,17} Seedlings are also sensitive to competition from weeds.⁵

Cotton volunteers occur in areas where cotton is cultivated and may occur due to spilling during transport or when feeding cattle.⁶ There are reports that *G. hirsutum* and *G. herbaceum* are

* Day degrees (or heat units) are a measure of time and temperature required to reach a certain plant developmental stage. They are calculated based on the daily minimum and maximum temperature minus the threshold temperature for growth and development of cotton (12 °C): [(daily max. temperature - 12) + (daily min. temperature - 12)] / 2. The day degrees for each day are summed during the growing season. When the average daily temperature drops below the threshold temperature, the daily increment of day degrees is set to zero.

naturalised in some Southern European countries, e.g. Greece and Spain.^{18,19} COGEM is not aware of any reports on feral cotton populations in Northwestern Europe. Wild relatives of cotton (*Gossypium* spp.) do not occur in Northwestern Europe. Therefore, hybridisation with wild relatives cannot occur in Northwestern Europe.⁵

Conclusion: The Dutch climate is unsuited for cotton cultivation. In the Netherlands, feral cotton populations do not occur, and hybridisation with other species is not possible because no wild relatives of cotton are present.

3.2 Description of the introduced genes and traits

Cotton COT102 was developed using *Agrobacterium tumefaciens* mediated transformation, using the binary vector pCOT1.

Introduced genes	Encoded proteins	Traits
<i>vip3Aa19</i>	Variant of a native vegetative insecticidal proteins(Vip) class A, subclass a, (Vip3Aa19) originating from <i>Bacillus thuringiensis</i> strain AB88 ^{20,21}	Resistance against certain lepidopteran insects
<i>aph4</i>	Hygromycin B phosphotransferase, or aminoglycoside phosphotransferase 4-Ia (APH4) enzyme, originating from <i>Escherichia coli</i> strain K-12 ²²	Resistance against the antibiotic hygromycin B
For a detailed description of the introduced genes and traits, see references		

3.3 Molecular characterisation

Southern blot analyses show that cotton COT102 contains one copy of the insert at a single integration locus, and demonstrate the absence of backbone sequences.

The applicant determined the sequence of the cotton COT102 insert and adjacent flanking sequences. During integration of the COT102 T-DNA, two insertions and one deletion occurred. A fragment of 86bp was deleted from the cotton genome. At the 5' junction between the insert and the cotton genome, 4bp of DNA was inserted in the genome. At the 3' junction between the insert and the cotton genome, a sequence of 690bp was inserted. The applicant states that the 3' insertion does not align to the genomic sequence of the non-GM cotton cultivar, but is highly similar to other *Gossypium* sequences. According to the applicant, no endogenous gene or regulatory element is disrupted at the insertion site.

The applicant also screened the junctions between the T-DNA insert and the flanking cotton genomic DNA as well as the entire insert (from stop to stop codon) for potential newly created open reading frames (ORFs). According to the applicant, no significant sequence similarities between the putative products of these ORFs and any known or putative allergenic or toxic proteins were detected in these bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.²³ The Syngenta toxin database that was used to identify toxic protein similarities was created from the NCBI Entrez® Protein Database (2016).²⁴ The COGEM notes that specific information about the selection criteria used to create the Syngenta database is lacking.

Conclusion: The molecular characterisation of cotton COT102 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of cotton COT102. The introduced traits do not give reason to assume that COT102 has an altered fitness compared to conventional cotton. COGEM is of the opinion that there are no indications that cotton COT102 will be able to survive or establish in the Dutch environment.

Conclusion: Cotton COT102 does not have an 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, RIKILT carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, RIKILT) 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^{25,26} but considers the current GS plan adequate for import and processing of cotton COT102.

6. Overall conclusion

COGEM is of the opinion that import and processing of cotton COT102 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. Antibiotic resistance genes and public perception

COT102 expresses the antibiotic resistance gene *aph4* which confers resistance to hygromycin B. The presence of antibiotic resistance genes in GM plants is controversial. By some antibiotic resistance genes in GM plants are perceived as a risk due to potential transfer of antibiotic

resistance genes to bacteria. However, so-called horizontal gene transfer between plants and bacteria has not been observed in field experiments. The *aph4* gene is present in bacteria in the environment, and this antibiotic is of minor importance to human medicine. Therefore, COGEM is of the opinion that the presence of *aph4* in GM plants poses a negligible risk to the environment.^{27,28}

In view of public perception, the presence of the *aph4* antibiotic resistance gene in COT102 may, however, be considered undesirable.

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