

Assessment of the Safety of Genetically Modified COT102 Cotton for Food and Feed Uses (RP2148)

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FSA Research and Evidence

The Food Standards Agency and Food Standards Scotland (FSA/FSS) received an application from Syngenta under assimilated regulation No. 1829/2003 for COT102 cotton. COT102 cotton has been modified by the addition of Vip3Aa19, an insecticidal protein from *Bacillus thuringiensis*, and APH4, which provides resistance to hygromycin and is used as an antibiotic resistance marker. The molecular characterisation of COT102 cotton confirmed the presence of a single transgenic insert, and bioinformatics analyses of this insert raised no safety concerns. Transgenic protein expression levels were determined and did not cause safety concerns. Genetic stability and phenotypic stability of the transgenic insert was also confirmed. The comparative assessment of COT102 cotton was deemed to be appropriate, and did not raise safety concerns. Studies on the Vip3Aa19 and APH4 proteins as expressed in COT102 cotton identified no safety concerns regarding toxicity or allergenicity, and no evidence was found that the genetic modification would change the overall allergenicity of COT102 cotton. The FSA/FSS agreed with the positive opinion of EFSA, and concluded that COT102 cotton is as safe as its conventional counterpart, with respect to its potential effects on human and animal health. This assessment represents the opinion of FSA and FSS.

This is a joint FSA and FSS publication.



1. Introduction

Following the submission of application RP2148 to the Food Standards Agency (FSA) under assimilated Regulation (EC) No. 1829/2003 from Syngenta, the Food Standards Agency (FSA) and Food Standards Scotland (FSS) assessed the application for genetically modified COT102 cotton. The scope of the application is for the import, processing, and food and feed use of COT102 cotton. The scope does not include cultivation of COT102 cotton in GB.

To ensure the regulatory systems of the FSA/FSS are risk proportionate, and resources are used effectively, the FSA/FSS have used the evidence submitted by the applicant and other information in the public domain, including an EFSA Scientific Opinion, to provide a summary assessment of the evidence of safety presented in this report.

The EFSA Scientific Opinion (EFSA, 2023) has been reviewed by FSA/FSS risk assessors. It has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

Although primarily used worldwide for its lint, cotton can also be used as food and/or feed. The raw, unprocessed cottonseed can be fed to ruminants, or the seed can be processed into oil for human consumption. Cottonseed oil has been in use since the 19th century and is considered a high-quality oil. Four species of cotton (*Gossypium* L.) can be cultivated; however, the most commonly cultivated, *Gossypium hirsutum*, provides over 90% of worldwide cotton production. Cotton production within Europe is limited to southern Europe, with Greece contributing approximately 80% of the area of cotton cultivation in Europe.

Cotton naturally contains some compounds which can have adverse effects on human and animal health. Gossypol is a polyphenol used by the plant as a natural defence compound against pests and disease. The gossypol content of raw cottonseed can vary between commercial

varieties; however, whole cottonseeds are not normally used as animal feed in the EU and the UK. Heat and moisture processing of cotton seeds can reduce the toxicity of gossypol. Cotton also contains several cyclopropenoid fatty acids (CPFAs) which can be present in oils derived from the cottonseed. Processing of cottonseed oil for human consumption removes the CPFAs; however, they can be present in animal feeds derived from cotton.

COT102 cotton is modified by the addition of Vip3Aa19, an insecticidal protein derived from Vip3Aa1, a protein from *Bacillus thuringiensis*, and APH4. Vip3Aa19 has insecticidal activity against several lepidopteran pests, including cotton bollworm and fall armyworm. APH4 provides resistance to hygromycin and is used as an antibiotic resistance marker.

This assessment represents the opinion of the FSA and FSS.

2. Details of other regulators opinions

2.1. Molecular characterisation

COT102 cotton was developed by *Agrobacterium*-mediated transformation of cotton (*Gossypium hirsutum*) hypocotyls with vector pCOT1. A non-oncogenic helper plasmid (pEHA101) was also used. The T-DNA region of pCOT1 consists of two expression cassettes, the *vip3Aa19* expression cassette, consisting of the promoter from the actin-2 gene from *Arabidopsis thaliana*, the codon-optimised *vip3Aa19* gene from *B. thuringiensis*, and the terminator from the nopaline synthase gene from *A. thaliana*; and the *aph4* expression cassette, comprising the promoter from the *ubiquitin-3* gene from *A. thaliana*, the *aph4* gene from *E. coli*, and the terminator from the nopaline synthase gene from *A. thaliana*.

Southern blot analysis was used by the applicant to confirm that COT102 cotton contains a single transgenic insert, consisting of a single T-DNA copy with the same organisation as the pCOT1 plasmid. Southern blot analysis also confirmed the absence of any backbone vector sequences. Sequencing of the T-DNA region, as well as 5' and 3' flanking regions, determined the sequence was identical to that of the pCOT1 plasmid, with the exception of a 24 bp (base pair) deletion from the right border sequence and a 19 bp deletion from the left border sequence. Insertion of the T-DNA resulted in a 86 bp target site deletion, and the addition of 4 bp of filler DNA. An additional 690 bp are also present in the 3' flanking region.

Bioinformatics analysis of the insertion locus and flanking sequences did not indicate any endogenous genes were interrupted by the T-DNA insertion. Bioinformatics analyses found four open reading frames (ORFs) at the T-DNA insertion locus that had some sequence similarities with

putative allergens; however, they were all in a different reading frame to the *vip3Aa19* coding sequence and did not possess start codons. Therefore, they are all unlikely to be expressed.

Expression levels of the transgenic proteins were determined by enzyme-linked immunosorbent assay (ELISA) on material harvested from field trials conducted in the USA in 2018. Expression levels were determined in leaves, bolls, flowers, pollen, roots, fuzzy cottonseeds, squares, and whole plants. Of these, expression levels in fuzzy cottonseeds and pollen were deemed the most relevant. Expression levels of Vip3Aa19 in pollen were below the levels of detection (3.20 µg/g dw (dry weight)), and expression levels of APH4 in fuzzy cottonseeds were below the level of quantification (0.25 µg/g dw). The expression levels of Vip3Aa19 in fuzzy cottonseeds were 2.16 µg/g dw (± 0.470) and expression levels of APH4 in pollen were 10.2 µg/g dw (± 1.44).

The genetic stability of the transgenic insert was determined by Southern blot analysis and PCR analysis on DNA derived from multiple generations. All plants tested retained a single copy of the insert which was stably inherited. Phenotypic stability was assessed by measuring expression of the transgenic proteins in leaves and seeds of multiple generations. The expression of Vip3Aa19 and APH4 were consistent across generations.

EFSA concluded that the molecular characterisation of COT102 cotton, and any associated bioinformatics analyses, did not raise any safety concerns. The FSA/FSS agree with the conclusions of EFSA.

2.2. Comparative assessment

COT102 cotton was grown alongside the conventional comparator (01W34) and several non-GM reference varieties (LA 122, AM UA 45, UA 222, HQ212CT, UA 114 and HQ21 OCT) at multiple field trial sites within the commercial cotton growing regions of the USA in 2018. The identity of the seeds used were confirmed by PCR, and their viability was assessed in warm and cold germination tests. The soil and climatic conditions at the field trial sites were diverse and corresponded to optimal, near-optimal, and sub-optimal cotton cultivation conditions. EFSA concluded that the seeds used in the field trials were of sufficient quality, and the locations used reflected the receiving environments in which COT102 cotton would be grown.

Nine agronomic and phenotypic endpoints were assessed by the applicant, as well as abiotic stressors, diseases, and pest damage. Statistically significant differences between COT102 cotton and the conventional counterpart were observed for plant height, fruit body count, final stand count, and seed weight; however, equivalence with the non-GM reference varieties was demonstrated for all these endpoints.

Fifty-seven compositional constituents were analysed by the applicant; however, statistical analysis was only performed on 44 of these. Statistically significant differences were observed for 12 endpoints; however, equivalence was demonstrated for all, except acid detergent fibre (ADF).

EFSA considered these differences, taking into account the natural variability observed in commercial cotton varieties, and determined that none of the differences identified in the comparative assessment required further analysis, or raised any safety concerns. The FSA/FSS agree with this assessment, confirming that any differences observed between COT102 cotton and the conventional comparator do not raise safety concerns in a GB context.

2.3. Food/feed safety assessment

An overview of existing information was provided by the applicant as part of the food/feed safety assessment of genetically modified COT102 cotton, including an assessment of the substrate specificity of APH4 (Stogios et al., 2011), the history of safe use of the source organisms of the transgenic proteins (*B. thuringiensis* and *E. coli* strain K-12), and information on the structure, function, and mode of action of the newly expressed proteins.

The stability of the newly expressed proteins was determined using temperature assays, pH assays, and *in vitro* digestion assays on microbially-produced Vip3Aa19 and APH4. EFSA considered these recombinant proteins suitable surrogates for determining the safety of plant-produced Vip3Aa19 and APH4. Temperature assays revealed Vip3Aa19 was unstable at temperatures exceeding 65 °C, and APH4 was unstable at temperatures above 120 °C. There was no effect on the mass and immunoreactivity of Vip3Aa19 at pH 1.2 and 7.5, and maximum APH4 activity was measured at pH 8.5. Both Vip3Aa19 and APH4 were degraded by pepsin at pH 1.2 within 60 seconds.

2.3.1. Toxicological assessment

A toxicological assessment of the newly expressed proteins using a weight-of-evidence approach found no evidence for potential toxic effects for either protein. Updated bioinformatics analyses found no amino acid sequence similarities between Vip3Aa19 or APH4 to known toxins. Acute toxicity studies of Vip3Aa19 and APH4 showed no adverse effects. A 28-day toxicity study of APH4 also showed no adverse effects; however, a high mortality rate was observed in the test group, attributed to dosing effects. The FSA/FSS Panel note that the high mortality observed in the test group due to non-compound related dosing effects could affect the power of

the study. In addition, 90-day feeding studies of COT102 cotton found no treatment-related adverse effects in rats after being fed a diet containing 3% or 10% COT102 cotton meal.

2.3.2. Allergenicity assessment

A weight-of-evidence approach was also used to determine any potential allergenicity. Both *B. thuringiensis* and *E. coli* are not considered to be allergenic sources, and updated bioinformatics analyses of both newly expressed proteins found no sequence similarities to known allergens. No evidence was found to suggest that Vip3Aa19 and APH4 might be adjuvants at the levels expressed in COT102 cotton. An assessment of whether the newly expressed proteins could cause a coeliac disease response found no indications of safety concerns. Overall, there were no indications that Vip3Aa19 and APH4 may be allergenic, and there was no indication of a potential increase in allergenicity of food and feed derived from COT102 cotton, compared to conventional non-GM cotton.

2.3.3. Exposure assessment

The human and animal dietary exposure to Vip3Aa19 and APH4 were also estimated. No consumption data for cottonseed, cottonseed oil, or other cottonseed-derived products exists for EU consumers; however, cottonseed oil is likely to be the most relevant to human consumers. However, as refined, bleached, and deodorised (RBD) oil for human consumption contains no proteins, exposure to Vip3Aa19 and APH4 is minimal. Exposure via cottonseed flour cannot be excluded; however, this is not largely consumed in Europe (or the UK) at this time. Animal exposure to Vip3Aa19 and APH4 was estimated in various animal species using a worst-case scenario where 100% of cotton in the diet was COT102 cotton.

2.3.4. Nutritional assessment

As the intended trait of genetically modified COT102 cotton is protection against lepidopteran pests, no nutritional effects are expected. RBD oil is the most consumed cottonseed-derived product by humans; however, COT102 cotton may also enter the food chain through cottonseed linters, which are used in baked goods, snacks, and processed meats. However, the comparative assessment showed COT102 cotton is the same as the conventional counterpart, with the exception of a small change in ADF levels, which does not represent a nutritional concern. Cotton seeds are mainly used in ruminants due to the high levels of fibre, and the small change in ADF observed in the comparative assessments does not cause a nutritional concern.

2.3.5. Conclusions on the food/feed assessment

EFSA concluded that the newly expressed proteins in COT102 cotton, Vip3Aa19 and APH4, do not raise safety concerns for human and animal health. Bioinformatics analyses of the sequences of both proteins found no evidence of potential toxicity or allergenicity concerns, and the stability of the proteins to temperature, pH, and *in vitro* digestion did not raise safety concerns. EFSA evaluated the human and animal dietary exposure to Vip3Aa19 and APH4, and found no safety concerns. The comparative assessment confirmed that the only difference between COT102 cotton and the conventional counterpart was a small change in ADF, which does not represent a safety, or nutritional, concern. The FSA/FSS agree with all the conclusions of EFSA which are applicable to GB, and confirm that COT102 cotton does not raise any safety concerns in terms of human and animal health.

2.4. Environmental risk assessment

As the application does not cover cultivation, EFSA's environmental risk assessment (ERA) focussed on the accidental release of COT102 cotton into the environment, and exposure of micro-organisms to recombinant DNA from COT102 cotton. The establishment and survival of volunteer cotton plants in the EU is limited and transient, and it is unlikely that the traits inserted into COT102 cotton will affect the ability of the cotton plants to survive. In addition, there is no evidence that the genetic modifications made to COT102 cotton increase the likelihood of horizontal gene transfer to micro-organisms in the environment. Therefore, EFSA concluded that it is unlikely that COT102 cotton would differ from conventional cotton in its ability to persist under European conditions. The FSA/FSS agree with this conclusion, as COT102 is highly unlikely to be able to persist in GB environmental conditions.

3. Other regulator's opinion and conclusions

The EFSA GMO Panel concluded that COT102 cotton is as safe as its non-GM conventional comparator with respect to its potential effects on human and animal health, and the environment (EFSA, 2023).

4. Uncertainties and limitations

No specific uncertainties or limitations were flagged in the assessment by EFSA. The FSA and FSS did not identify further uncertainties or limitations to be considered for this assessment.

5. FSA & FSS conclusions for GB assessment

The FSA and FSS assessed the EFSA opinion for genetically modified COT102 cotton (EFSA, 2023) and confirmed that it is adequate and relevant for GB risk analysis.

The conclusions of the EFSA opinion have been reviewed in detail by FSA and FSS and are considered appropriate and consistent, including the uncertainties and limitations identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

The ERA of COT102 cotton has been completed by ACRE (Advisory Committee on Releases to the Environment) in terms of the GB environment. Their conclusions match those of EFSA, in that it is unlikely that COT102 cotton would differ from conventional cotton in its ability to persist under UK conditions.

[ACRE's advice](#) is available on the [GOV.UK](#) website.

6. Outcome of the assessment

The FSA and FSS have reviewed the applicant's dossier, supporting documentation, and most notably the EFSA opinion (EFSA, 2023), and consider that there is sufficient evidence to conclude the assessment of genetically modified COT102 cotton without obtaining further information or conducting a further risk assessment.

The FSA and FSS conclude that genetically modified COT102 cotton is as safe as its non-GM conventional comparator with respect to its potential effects on human and animal health, and the environment.

In making this assessment, the FSA and FSS were able to rely on sufficient scientific evidence to make a conclusion on safety with no further questions to the applicant, and therefore no further risk assessment activities are necessary.

Applicants provided sufficient relevant information as requested by the FSA and FSS.

The FSA and FSS review did not find any issues of divergence from the EFSA guidance (EFSA GMO Panel, 2011, 2015, 2017) or mutual approaches or new scientific issues for consideration.

There were no other specific issues that would require an assessment for the UK or the nations of the UK.

Abbreviations

Abbreviation	Definition
ACRE	Advisory Committee on Releases to the Environment
ADF	Acid detergent fibre
bp	Base pair
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ERA	Environmental risk assessment
EU	European Union
ORF	Open reading frame
PCR	Polymerase chain reaction
RBD	Refined, bleached, and deodorised
T-DNA	Transfer-deoxyribonucleic acid

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