U.S. FOOD & DRUG ADMINISTRATION

# Biotechnology Notification File No. 000163
# CFSAN Note to the File

**Date:** September 18, 2019

**From:** Patrick Cournoyer, PhD

**To:** Administrative Record, BNF No. 000163

**Subject:** Cotton with transformation event TAM66274 (TAM66274 cotton)

**Keywords:** Cotton; *Gossypium hirsutum*; cottonseed; gossypol; RNA interference (RNAi); neomycin phosphotransferase II (*nptII*) from *Escherichia coli* Tn*5*; delta-cadinene synthase (dCS); OECD unique identifier TAM-66274-5; Texas A&M University; Texas A&M AgriLife Research

## Summary

Texas A&M AgriLife Research (Texas A&M) has completed a consultation with the Food and Drug Administration (FDA) on food derived from TAM66274 cotton with reduced levels of gossypol in seed. Gossypol is a toxicant in cotton that limits uses of cottonseed from conventional cotton varieties in human food.[1] This document summarizes Texas A&M's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Texas A&M concludes:

- human food ingredients from TAM66274 cotton would not require premarket approval as food additives.
- gossypol content in seed from TAM66274 cotton is within the 450 ppm (mg/kg) limit for cottonseed-derived human food ingredients (25 FR 8368, September 1, 1960; 41 FR 19933, May 14, 1976; 21 CFR 172.894).
- a new protein or other substance that would require premarket approval as a food additive has not been introduced into human food from TAM66274 cotton.
- food from TAM66274 cotton is as safe as human food from other cotton and, except for the reduced gossypol trait, food from TAM66274 cotton is comparable to human food from other cotton.

[1] Cotton varieties have been bred (without the use of genetic engineering) to contain reduced levels of gossypol. Such cotton varieties are referred to as glandless, as they lack gossypol-containing glands throughout the plant. Gossypol protects cotton from pests, making glandless cotton varieties more susceptible to pests. The reduced gossypol trait in TAM66274 cotton is specific to seed and thus is not expected to significantly reduce pest resistance in other parts of the plant.

- roasted cottonseed kernels, raw cottonseed kernels,[2] cottonseed kernels, partially defatted cottonseed flour, defatted cottonseed flour, and cottonseed oil are appropriate common or usual names for ingredients derived from TAM66274 cotton for use in human food.[3]

We evaluated data and information supporting these conclusions and considered whether TAM66274 cotton raises other regulatory issues involving human food under the Federal Food Drug and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from TAM66274 cotton.

## Subject of the Consultation

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| **Crop:** | Cotton |
| **Designation:** | TAM66274 |
| **Trait:** | Decreased levels of gossypol in seed |
| **Developer:** | Texas A&M University |
| **Original submission received:** | September 25, 2017 |
| **Amendment received:** | February 16, 2018; June 2, 2019 |
| **Intended use:** | General uses customary for conventional cotton, and uses authorized for glandless cotton in human food[4] |
| **Expression cassette 1:** | RNAi gene suppression cassette intended to suppress expression of *delta-cadinene synthase* (*dCS*). Expression of the cassette is driven by the seed-specific alpha-globulin B promoter from cotton. Delta-cadinene synthase is a key enzyme in gossypol biosynthesis. |
| **Expression cassette 2:** | *nptII* gene encoding neomycin phosphotransferase II (NPTII) derived from *Escherichia coli transposon Tn5*. NPTII imparts antibiotic resistance for use as a selectable marker. |
| **Transformation method:** | *Agrobacterium*-mediated transformation |

[2] The food additive regulation 21 CFR 172.894 authorizes the use of raw glandless cottonseed kernels in hard candy where the kernel temperature during cooking will exceed 250°F for not less than 5 minutes. The regulation authorizes the use of raw glandless cottonseed kernels by food manufacturers; however, it does not authorize the use cottonseed kernels in raw form for direct consumption. Therefore, "cottonseed kernels" is an appropriately descriptive term to include in the ingredient statement on labels of food products made with raw cottonseed kernels that have subsequently been cooked.

[3] CFSAN's Office of Nutrition and Food Labeling, Food Labeling and Standards Staff considers these names to be sufficiently descriptive terms for food ingredients from TAM66274 cotton.

[4] Roasted glandless cottonseed kernels are food additives authorized for use in baked goods, as a snack food, and in soft candy; raw glandless cottonseed kernels are authorized for use in hard candy that is subsequently cooked (41 FR 19933, May 14, 1976; amending 21 CFR 121.1019, now 21 CFR 172.894).

# Molecular Characterization

## Confirmation of intended genetic change

Texas A&M used Southern blot analysis to confirm the presence of a single, intact DNA insertion. The insertion and flanking cotton DNA were amplified using polymerase chain reaction (PCR) and sequenced to confirm the integrity of the inserted DNA.[5] Texas A&M used Southern blot analysis with a series of ten overlapping probes spanning the vector backbone to confirm the absence of vector backbone DNA in TAM66274 cotton.

## Stability over multiple generations

Texas A&M performed Southern blot analysis on three breeding generations of TAM66274 cotton, finding consistent hybridization patterns in each generation. Texas A&M observed the expected 3:1 Mendelian segregation ratio for the trait, evidenced by light-colored glands associated with the low gossypol phenotype, and by PCR analysis showing presence of the insertion.

# Introduced Protein: Neomycin phosphotransferase II (NPTII)

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| **Source** | *Escherichia coli* transposon Tn*5* |
| **Protein description** | Aminoglycoside phosphotransferase[6] |
| **Intended function** | Phosphorylation of aminoglycoside antibiotics |
| **Intended trait** | Resistance to aminoglycoside antibiotics, for use as a selectable marker during genetic engineering |

## NPTII safety assessment

FDA conducted a comprehensive safety review and authorized as a food additive the use of NPTII (referred to as aminoglycoside 3′ phosphotransferase II in FDA's regulations) as a selectable marker in certain genetically engineered plant species, including cotton (59 FR 26700, May 23, 1994; 21 CFR 173.170 for uses in human food).

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[5] Texas A&M used the flanking cotton DNA sequences to identify the insertion site in the cotton reference genome and found that the insertion replaced 44 base pairs of cotton DNA in a putative alpha-hydrolase gene. Texas A&M measured alpha-hydrolase gene expression in TAM66274 cotton using reverse transcriptase PCR and found no major differences relative to the non-genetically engineered control cultivar Coker 312. Based on this finding, and based on phenotypic, agronomic, and compositional evaluation, Texas A&M concludes that this change to endogenous cotton DNA had no significant impact on plant metabolism, growth, or development.

[6] Texas A&M notes that the *nptII* gene in TAM 66274 contains nucleotides from the *nos* gene of *A. tumefacien* resulting in amino acid replacements at the N-terminus of the NPTII protein and that the modified gene was used previously in several EPA-regulated products and does not affect the safety of the NPTII protein.

## Cottonseed Allergenicity

Texas A&M reviewed the scientific literature on allergic sensitivity to cottonseed protein, including case reports of individuals who experienced allergic reactions. Texas A&M considers the occurrence of cottonseed allergy to be rare. Texas A&M states that no evidence suggests TAM66274 cotton would be more allergenic than other cotton varieties.[7]

## Characterization of the Intended Trait (Reduced Gossypol)

Texas A&M measured levels of gossypol in seed from TAM66274 cotton and from the non-genetically engineered variety Coker 312 (control) using standard industry methods.[8] Mean levels of free gossypol in TAM66274 cotton, expressed on a dry weight (DW) basis, were 300 ppm in 2014 field trials and 260 ppm in 2015 field trials.[9] Levels in the control were 7,770 ppm in 2014 and 8,300 ppm in 2015. Levels of free gossypol in TAM66274 cotton did not exceed the limit of 450 ppm for cottonseed-derived ingredients for human consumption (21 CFR 172.894).

## Human Food Nutritional Assessment

To ensure the absence of unintended changes in the levels of other components relevant to safety or nutrition, Texas A&M analyzed seed from TAM66274 cotton and from Coker 312 (control) for proximates (protein, crude fat, ash, and total carbohydrates by calculation), moisture, calories, fiber (crude fiber, total dietary fiber, acid detergent fiber, and neutral detergent fiber), amino acids, fatty acid profile including cyclopropenoid fatty acids, minerals (copper, iron, manganese, zinc, calcium, magnesium, phosphorus, potassium, and sodium), alpha-tocopherol, phytic acid, and mycotoxins. Texas A&M analyzed seed from two growing seasons using validated analytical methods.[8]

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[7] FDA notes that the entry of TAM66274 cotton into the human food supply could result in increased consumer exposure to cottonseed protein. Sensitive individuals can avoid foods containing allergens through product labeling.

[8] Texas A&M analyzed the composition of seed from TAM66274 cotton and from the control, Coker 312, grown in field trials at three locations in the United States in 2014, and at five locations in the United States in 2015.

[9] Texas A&M measured free gossypol using an aniline-based method. Texas A&M also measured levels of total (bound and free) gossypol using an aniline-based method and an HPLC-based method. The aniline-based method found total mean gossypol values in TAM66274 cotton of 440 ppm in 2014 and 420 ppm in 2015; this method can overestimate gossypol content because it also detects other terpenoids. The HPLC-based method, which is more specific, found total mean gossypol values of 370 ppm in 2014 and 300 ppm in 2015.

Texas A&M observed minor differences between TAM66274 and the control in the levels of total dietary fiber, individual fatty acids, cyclopropenoid fatty acids, and alpha-tocopherol in seed from both growing seasons. However, with the exception of the intended trait (reduced gossypol), levels of all components in TAM66274 cotton were within ranges for cottonseed reported in the scientific literature for conventional varieties with a history of safe use in food. Texas A&M concludes that the observed compositional differences would not impact the nutritional value of food from TAM66274 cotton.

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