Health Canada has notified Syngenta Seeds Canada Inc. that it has no objection to the sale of food derived from Insect Resistant Cotton COT67B. The Department conducted a comprehensive assessment of this cotton event according to its Guidelines for the Safety Assessment of Novel Foods. These Guidelines are based upon internationally accepted principles for establishing the safety of foods with novel traits.

The following provides a summary of the notification from Syngenta Seeds Canada and the evaluation by Health Canada and contains no confidential business information.

1. Introduction

Syngenta Seeds Canada developed Insect Resistant Cotton COT67B by modifying commercial cotton line Coker 312 using recombinant DNA techniques to introduce the coding sequence (flcry1Ab) for the novel protein FLcry1Ab derived from the common soil bacterium Bacillus thuringiensis subspecies kurstaki strain HD-1 (Btk). FLcry1Ab is an insecticidal protein effective against lepidopteran pests. Commercial cotton line Coker 312 was also modified through the introduction of the coding sequence for the antibiotic resistance gene hygromycin B phosphotransferase (aph4) derived from E.coli strain K12. This gene was introduced to act as a selectable marker for the integration of desired trait FLcry1Ab. This marker was integrated using a separate vector to that containing FLcry1Ab and Syngenta removed the marker gene from the final COT67B line through selective breeding.

The safety assessment performed by Food Directorate evaluators was conducted according to Health Canada's Guidelines for the Safety Assessment of Novel Foods. These Guidelines are based on harmonization efforts with other regulatory authorities and reflects international guidance documents in this area (eg., Codex Alimentarius). The assessment considered: how Insect Resistant Cotton COT67B was developed; how its composition and nutritional quality compares to traditional cotton varieties; and the potential for the presence of any toxicants, anti-nutrients, or allergens. Syngenta has provided data which demonstrates that Insect Resistant Cotton COT67B is as safe and nutritious as conventional cotton varieties sold in Canada.

The Food Directorate has a legislated responsibility for pre-market assessment of novel foods and novel food ingredients as detailed in Division 28 of Part B of the Food and Drug Regulations (Novel Foods). Foods
derived from Insect Resistant Cotton COT67B are considered novel foods under the following part of the definition of novel foods: “c) a food that is derived from a plant, animal or microorganism that has been genetically modified such that

(i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism.

2. Development of the Modified Plant

COT67B was developed through disarmed *Agrobacterium*-mediated transformation of cotton cultivar Coker 312 using a two T-DNA system. The insecticidal gene (*flcry1Ab*) and the selectable marker gene (*aph4*) were delivered as separate T-DNA inserts via separate plasmids (pNOV4641 and pNOV1914, respectively), and thus independently inserted into separate loci in the cotton genome.

The *flcry1Ab* gene cassette in plasmid pNOV4641, contained the actin-2 (ACT2) promoter from *Arabidopsis thaliana*, a synthetic copy of the coding region of *flcry1Ab* from *Bacillus thuringiensis* strain HD-1 and the termination sequence of the nopaline synthase gene from *Agrobacterium tumefaciens* (Nos 3’).

The *aph4* gene cassette in plasmid pNOV1914, contained the promoter plus the first intron of the ubiquitin-3 gene of *Arabidopsis thaliana*, the coding region of *aph4* from *E. coli* strain K12 and the termination sequence of the nopaline synthase gene from *Agrobacterium tumefaciens* (Nos 3’).

After selection on media containing hygromycin, only recombinant cells containing the *aph4* gene survive. Regenerated plants containing both the *flcry1Ab* and *aph4* inserts were selected for subsequent conventional breeding. Resulting progeny are genetically selected for the presence of the *flcry1Ab* cassette, and the absence of the *aph4* cassette. The final COT67B line contains the *flcry1Ab* cassette from the T-DNA of pNOV4641, and does not contain the *aph4* cassette of the T-DNA of pNOV1914.

3. Characterization of the Modified Plant

Southern blot analysis of COT67B cotton demonstrated the insertion of a single copy of the *flcry1Ab* gene cassette in the cotton genome at a single locus. Southern blot analysis also demonstrated the integrity of the *flcry1Ab* gene and associated regulatory elements. Southern blot analysis also demonstrated, as expected, the absence of any plasmid pNOV4641 derived sequences outside the T-DNA region, such as the spectinomycin resistance gene found in the plasmid backbone. The elements contained in the cassette were shown to be stable with no rearrangements through Southern blot and sequence analysis. These analyses of the insert show that the cassette is entirely integrated into the genome and that all the elements are intact.

Southern blot analysis of COT67B cotton also demonstrated, as expected, the absence of the *aph4* gene cassette. Additionally, the petitioner provided Southern blot analysis demonstrating the absence of any plasmid pNOV1914 derived sequences outside the T-DNA region, such as the spectinomycin resistance gene found in the plasmid backbone. The evidence presented by the petitioner demonstrates that the antibiotic resistance marker gene, *aph4*, has successfully been bred out of COT67B.

The petitioner has provided data for five generations of COT67B cotton. The results of Southern blot analysis and segregation data demonstrated the stability of COT67B at the genomic and phenotypic levels.

Due to the low levels of protein expressed in plants, the petitioner has conducted the toxicological assessment using VIP3A protein expressed in and purified from *E. coli*. To ensure that the results of the toxicological studies are applicable to the protein expressed in COT67B, equivalence studies (i.e., SDS PAGE, Western blot analysis, MALDI-TOF MS, glycosylation analysis, and insecticidal activity analysis) were conducted to confirm that the protein produced in *E. coli* used for toxicology studies is representative of the protein produced in the modified cotton plant. For FLcry1Ab, the data presented indicated that the proteins can be considered equivalent.

4. Product Information

Insect Resistant Cotton COT67B differs from conventional cotton by the insertion of the novel gene *flcry1Ab* and associated regulatory elements. The insertion of this gene results in the expression of the novel protein FLcry1Ab. The expression of FLcry1Ab confers resistance to lepidopteran pests.

FLcry1Ab protein expression levels are determined by enzyme-linked immunosorbent assay (ELISA) in several cotton tissues collected from four field trials conducted in the United States in 2004. Quantifiable concentrations of FLcry1Ab were detected on all COT67B plant tissues except fibre and nectar. Protein quantities for the tissues were calculated on a microgram (μg) per gram (g) fresh weight (fwt) basis. The range of FLcry1Ab expressed in the whole plant from US-grown COT 67B was from 6.99 - 19.41 μg/g fwt, with a mean value of 10.85 μg/g fwt.

Human consumption of cotton products is limited to refined cottonseed oil and cellulose from processed linters, and is the primary interest for human food safety.

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assessment. The levels of FLCry1Ab in the COT67B processed fractions of once-refined oil and cotton linters are <LOD and 9.65 ug/g, respectively.

5. Dietary Exposure

Cottonseed from Insect Resistant Cotton COT67B will be used in applications similar to those derived from other cotton varieties. The use of cotton for human consumption is limited to refined cottonseed oil and linters, short fibres which are processed as a source of food grade cellulose and found in products such as bologna and sausage casings. The use of these linters in human food is routine, safe and well documented.

6. Nutrition

The nutrient data for this submission was obtained from COT67B and control Coker 312 cotton plants grown in 2004, at four different locations in the US representing the agriculture regions where these varieties would typically be cultivated. A randomized complete block with four replicate plots at each location was used.

Harvested cottonseed was measured for 41 compositional analytes: proximates (moisture, protein, fat, carbohydrate, acid detergent fibre, neutral detergent fibre and total dietary fibre), minerals (calcium and phosphorus), fatty acids, vitamin E, amino acids, and anti-nutrients (free and total gossypol, and cyclopropenoid fatty acids (sterculic, malvalic, and dihydrosterculic acid)).

Of the 41 analytes measured, six showed a statistically significant difference between COT67B and control: calcium (higher in COT67B), palmitic (lower), stearic (higher) and oleic acids (higher), dihydrosterculic acids (lower) and vitamin E (higher). In all cases the differences between the two varieties were very small (e.g., for calcium, COT67B contained 1.5% Ca, while the control had 1.4%). The level of each of these six analytes was within the published range for the respective analyte in cottonseed.

An additional small study to examine the levels of antinutrients (gossypol and cyclopropenoid fatty acids) in processed fractions, refined oil and cottonseed meal was also conducted. In this study, two samples each of refined oil and cottonseed meal were analysed. Although the data was not statistically analysed, the levels of antinutrients in COT67B processed fractions were similar to those of the control, Coker 312.

7. Toxicology

The novel gene present in COT67B cotton was isolated from a microorganism with no known pathogenicity to humans. FLCry1Ab was shown not to be acutely toxic to mice when administered by oral gavage at doses of 1830 mg/kg bw and no clinical signs were observed. The protein shows no amino acid sequence homology with any known human toxins. Furthermore, the petitioner has provided data demonstrating that the protein is rapidly degraded in simulated gastric and intestinal fluids.

The primary human food uses of cotton are limited to refined cottonseed oil and cottonseed linters. Both refined cottonseed oil and cottonseed lint are essentially devoid of protein. Therefore, anticipated human dietary exposure to the novel protein, FLCRY1Ab, in COT67B cotton through the direct consumption of cotton products would be negligible.

Cotton contains two categories of naturally occurring toxins: cyclopropenoid fatty acids (e.g., stereulic acid, malvalic acid and dihydrosterculic acid) and terpenoid phytalexins (e.g., gossypol). The levels and distribution of cyclopropenoid fatty acids were comparable in the parent and transgenic plants, except in the case of dihydrosterulic acid. In this case the transgenic, COT67B was found to have lower levels of the toxin than the parental control. There are no significant differences in the levels of gossypol in cottonseed from event COT67B and its non-trangenic parent. Therefore, in this regard, the novel plant does not raise any additional health concerns when compared to the non-transgenic parent from which it was derived.

The novel protein, FLCry1Ab, is considered unlikely to be an allergen since it does not share the characteristics of proteins that are food allergens. Unlike many food allergens, the novel proteins constitute a negligible amount of the total protein in food. No significant homology was found between the amino acid sequences of FLCry1Ab and those of any known allergen. Both novel proteins are rapidly degraded in simulated gastric fluid, as detected by Western blot analysis, suggesting that the FLCry1Ab protein would be digested in the mammalian digestive tract.

The primary human food uses of cotton are limited to refined cottonseed oil and cottonseed linters. Both refined cottonseed oil and cottonseed lint are essentially devoid of protein. Therefore, anticipated human dietary exposure to the novel protein, FLCRY1Ab in COT67B cotton, through the direct consumption of cotton products, would be negligible.

8. Conclusion

Health Canada’s review of the information presented in support of the food use of Insect Resistant Cotton COT67B concluded that derived food products do not
raise concerns related to safety. Health Canada is of the opinion that Insect Resistant Cotton COT67B is similar to regular conventional commodity cotton in terms of being an acceptable food source.

Health Canada’s opinion deals only with the human food use of Insect Resistant Cotton COT67B. Issues related to the environmental safety of Insect Resistant Cotton COT67B in Canada and its use as livestock feed have been addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.