

Canadian Food  
Inspection AgencyAgence canadienne  
d'inspection des aliments

Canada

Français	Contact Us	Help	Search	Canada Site
Table of Contents	What's New	Acts and Regulations	Site Map	
Food Safety	Animal Health	Plant Protection	Corporate Affairs	
Quick Pick By Commodity / Key Topic <input type="text"/>				

- [Main Page - Plant Biosafety Office](#)
- [Main Page - Plant Health and Production Division](#)
- [The Regulation of Plants With Novel Traits in Canada](#)
- [Guidelines for the Environmental Release of Plants with Novel Traits within Confined Field Trials in Canada \(Dir2000-07\)](#)
- [Decision Documents](#)
- [Contacts](#)

Plant Health and Production Division,  
Plant Biosafety Office

## Decision Document 96-14: Determination of Environmental Safety of Bollgard™ Insect Resistant Cotton (*Gossypium hirsutum* L.)

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines [Dir95-03 Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed](#) and [Dir94-08 Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits](#).

Agriculture and Agri-Food Canada, specifically the Feed Section of the Plant Health and Production Division, with input from the Plant Health Risk Assessment Unit, AAFC, and advice from the Plant Biotechnology Office, AAFC has evaluated information submitted by Monsanto Canada. These plants were transformed with genes conferring resistance to lepidopteran pests including the cotton bollworm, the tobacco budworm and the pink bollworm and resistance to kanamycin as a selectable marker. AAFC has determined that feed derived from these plants with novel traits should not pose a concern to environmental or livestock feed safety.

Feed use, as cottonseed and meal of the Bollgard™ cotton lines 531, 757 and 1076 and other *G. hirsutum* lines derived from them, but without the introduction of any other novel trait, is therefore authorized.

### Table of Contents

- I. [Brief Identification of the Plants with Novel Traits \(PNTs\)](#)
- II. [Background Information](#)
- III. [Description of the Novel Traits](#)
  1. [Insect Resistance](#)
  2. [Kanamycin Resistance](#)
  3. [Development Method](#)
  4. [Stable Integration into the Plant's Genomes](#)
- IV. [Assessment Criteria for Environmental Safety](#)
  1. [Potential of the PNTs to Become Weeds of Agriculture or Invasive of Natural Habitats](#)
  2. [Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive](#)
  3. [Altered Plant Pest Potential](#)
  4. [Potential Impact on Non-Target Organisms](#)
  5. [Potential Impact on Biodiversity](#)
  6. [Potential for Development of Insect Resistance to the PNTs](#)
- V. [Nutritional Assessment Criteria as Livestock Feed](#)
  1. [Anti-Nutritional Factors](#)
  2. [Nutritional Composition of the PNTs](#)

## VI. Regulatory Decision

---

### I. Brief Identification of the Plants with Novel Traits (PNTs)

<i>Designation(s) of the PNT:</i>	Bollgard <sup>TM</sup> cotton lines 531, 757, 1076
<i>Applicant:</i>	Monsanto, Canada
<i>Plant Species:</i>	Cotton ( <i>Gossypium hirsutum</i> L.)
<i>Novel Traits:</i>	Resistance to lepidopteran pests (cotton bollworm pink bollworm, tobacco budworm); kanamycin (antibiotic) resistance
<i>Trait Introduction Method:</i>	<i>Agrobacterium tumefaciens</i> -mediated transformation
<i>Proposed Use of PNT's:</i>	Production of cotton for fibre, cottonseed and cottonseed meal for livestock feed, and cottonseed oil for human consumption. These materials will be grown outside Canada, in the usual production areas for cotton. Cottonseed and cottonseed meal will be imported into Canada for livestock feed use only.

### II. Background Information

Monsanto has developed three cotton lines resistant to the larvae of the lepidopteran insect pests which are specific to cotton. These cotton lines, referred to as Bollgard<sup>TM</sup> cotton (lines 531, 757, and 1076) in the present document, will provide an alternative method for the control of cotton bollworm, pink bollworm and tobacco budworm, thus resulting in a potential reduction in the use of chemical foliar insecticide sprays.

The development of the Bollgard<sup>TM</sup> cotton lines was accomplished with recombinant DNA technology, resulting in the insertion of two bacterial genes into the Coker 312 cotton variety. A gene coding for a protein insecticidal to lepidopteran caterpillars was introduced along with a gene conferring resistance to the antibiotic, kanamycin. The antibiotic resistance gene is of no agricultural interest but the trait was used to select transformed from non-transformed plants during the development phase.

Field tests for the Bollgard<sup>TM</sup> cotton lines took place at 175 locations in 14 states in the United States. The lines have received complete approval for commercialization in the United States. Health Canada has determined that cottonseed oil derived from this cotton is substantially equivalent to that derived from currently commercialized cotton (April 19, 1996).

Monsanto has provided data on the identity of the Bollgard<sup>TM</sup> cotton lines, a detailed description of the modification method, data and information on the gene insertion sites, copy numbers and levels of expression in the plant, the role of the inserted genes and regulatory sequences in donor organisms, and full nucleotide sequences. The novel proteins were identified, characterized, and compared to the original bacterial proteins, including an evaluation of their potential toxicity to livestock and non-target organisms with particular attention to beneficial arthropods. Relevant scientific publications were also supplied.

Agronomic characteristics such as yield, boll size, plant vigour, growth, morphology, germination and flowering, were compared to those of unmodified cotton counterparts. Stress adaptation was evaluated, including susceptibility to various pests and pathogens. Processing qualities of cotton lint such as micronaire, length, strength and elongation were compared. The observed variability was within the range of inherent

variability of cotton varieties and was not attributed to the inserted genes.

Data to support the suitability of cottonseed and cottonseed meal from the Bollgard<sup>TM</sup> cotton lines as livestock feed was provided. Proximate analyses including crude protein, crude fat, crude fibre, ash and gross energy were supplied.

Since the insect resistant cotton will not be grown in Canada, Monsanto will not be required to institute a pest management strategy.

The Feed Section of the Plant Health and Production Division, with input from the Plant Health Risk Division, AAFC and advice from the Plant Biotechnology Section has reviewed the above information. The following assessment criteria, as described in the regulatory directives [Dir94-08](#) and [Dir95-03](#) were used to determine the suitability as livestock feed and the environmental safety of feed from these plants with novel traits:

- potential impact on livestock
- potential impact on livestock nutrition
- potential impact of the PNTs or their gene products on non-target species, including humans
- potential for the PNTs to become plant pests,
- potential of the PNTs to become weeds of agriculture or to be invasive of natural habitats,
- potential impact on biodiversity
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive.

### III. Description of the Novel Traits

#### 1. Resistance to the Lepidopteran Insects

- *Bacillus thuringiensis* subsp. *kurstaki* HD-73 (B.t.k.) is a common gram-positive soil-borne bacterium. In its spore forming stage, it produces a CryIA(c) insecticidal, crystal protein that is non-toxic to humans, other vertebrates, and beneficial insects. This and similar proteins are used as environmentally acceptable foliar insecticides against lepidoptera.
- The plant expressed toxin is similar to the *Bacillus thuringiensis* var. *kurstaki* HD-73, insecticidal, crystal protein. This protein is insecticidal to Lepidoptera larvae after cleavage to an approximately 600 amino acid, bio-active, trypsin resistant core. Insecticidal activity is believed to depend on the binding of the active fragment to specific receptors on the insect, midgut epithelial cells, forming a pore which disrupts osmotic balance and eventually results in cell lysis then death. Current knowledge indicates that only sensitive insects possess such receptors. Specific Lepidopteran pests of cotton, sensitive to the protein are: cotton bollworm, tobacco budworm and pink bollworm.
- The inserted gene codes for an insecticidal protein similar to a full length Cry IA(c) protein with some minor changes arising from the design of the gene. The complete sequence of the T-DNA with the genetic endpoints and the deduced amino acid sequence was included in the submission. The encoded Cry1A(c) plant produced protein is 99.4% homologous to the native protein from B.t.k.-HD73.
- The *cryIA* gene is linked to a strong constitutive promoter. Protein production (was quantified for the three lines. Mean CryIA(c) expression in line 531 was 1.56 µg protein/g and 0.86 µg protein/g fresh weight in leaf and seed tissue respectively. In the whole plant the Cry1A(c) protein averaged 0.044 µg protein/g fresh weight, (25 µg/ plant) for a total of about 1.44 g/ acre. Expression varied approximately 3 fold over the growing season, with the levels peaking late in the season. Expression of the CryIA(c) protein was much higher in line 757. Average values were 12.6 µg protein/g fresh weight and 9.9 µg protein/g fresh weight in leaves and seeds respectively. Expression varied less than 3 fold over the season and peaked early in the season in leaf tissue. Whole plant amounts were 1.1 µg protein/g fresh weight (200 µg/plant) for a total of 12.2 g/ acre. Line 1076 produced the cry1A(c) protein at an average of 12.2 µg protein/g fresh weight and 12.7 µg protein/g fresh weight in leaves and seed respectively. Gene expression over the growing season varied less than 5 fold, with the highest concentration occurring early in the season. Whole plant totals were 1.1 µg protein/g fresh weight (389 µg/plant) for a total of 23.3 g/ acre. (Calculations per acre based on 60000 plants/acre). Expression in pollen and nectar was negligible in all 3 lines.
- Biological activity of the plant expressed protein was compared to that of the native *Btk*-HD-73. The insecticidal protein was shown to be active on only lepidopteran species, even when fed at levels that

were well above the concentrations that were effective on target species. Host range specificity was similar for the native and synthetic proteins Tobacco hornworm was the most sensitive species and tobacco budworm, corn earworm and European cornborer were relatively less sensitive.

- The plant expressed protein degraded rapidly (less than 30 seconds) in simulated gastric fluid (pH 1.2) and in intestinal fluid (pH 7.5). A bioactive tryptic core remained after 21 hours in simulated intestinal fluid. Degradation of the protein was determined by western blot analysis and by insect bioactivity. Degradation of the B.t.k-HD-73 protein in soil proceeded with a half life of 41 days as determined by the insecticidal activity on tobacco budworm.
- Protein allergens are normally resistant to digestion unlike the Cry1A(c) protein, which is rapidly metabolized. Unlike many known allergens the insecticidal protein is not glycosylated. Screening against a database of known allergens for both the full length protein and the tryptic fragment revealed no significant homology to any known allergenic protein.

## 2. Kanamycin Resistance

- Kanamycin is an aminoglycosidic antibiotic that binds to bacterial ribosomes thus disrupting normal protein synthesis and killing the bacterial cell.
- The kanamycin-resistance gene, isolated from the bacterium *E. coli*, codes for an enzyme that phosphorylates kanamycin, thereby preventing it from binding to ribosomes and rendering the cells resistant.
- The gene is linked to a constitutive promoter. Expression levels for the NPT II protein in Line 531 were 3.14 µg protein/g fresh weight and 2.45 µg protein/g fresh weight in leaf and seed tissues respectively. Expression varied about 2 fold over the season. In line 757 NPT II expression was 6.9 µg protein/g fresh weight and 3.3 µg protein/g fresh weight in leaves and seeds respectively. For line 1076 the NPT II expression was 16.3 µg protein/g fresh weight and 7.9 µg protein/g fresh weight in leaves and seed respectively. Gene expression varied 2 to 3 fold over the growing season for line 757 and line 1076.
- This protein is ubiquitous in the environment. It degrades rapidly in vitro in simulated mammalian gastric and intestinal fluids.
- The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENE BANK DNA database.
- The full nucleotide sequence and corresponding amino acid sequence were provided.
- There was no detectable expression of the streptomycin/spectinomycin resistance marker used for selection of bacterial transformants during the development process. The gene is driven by a bacterial promoter and its lack of expression in the transformed plant was confirmed by specific ELISA.

## 3. Development Method

- Coker line 312 cotton was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector. The vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and genes coding for lepidopteran and kanamycin resistance were inserted. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was demonstrated in the Bollgard<sup>TM</sup> cotton.

## 4. Stable Integration into the Plant's Genome

- The inserted T-DNA, including the partial genes, was well characterized by restriction enzyme analysis, for all 3 lines. The genes were stable across 4 generations, for lines 531 and 1076 as shown from data collected at 1 site. Line 757 has an intact T-DNA at one site and a partial T-DNA inserted at another site in the genome. The 2 insertions were inherited in a linked Mendelian manner. Stability for this line was tested and confirmed across 2 generations.

## IV. Assessment Criteria for Environmental Safety

### 1. Potential of the PNTs to Become Weeds of Agriculture or Be Invasive of Natural Habitats

Cotton (*G. hirsutum*) is a member of the family Malvaceae. It is a perennial species cultivated as an annual and grown in the United States, mostly in areas from Virginia southward and westward to California. Cotton is not considered a weed pest in the regions where it is grown. It is not grown in Canada as it is not adapted to environmental conditions found at these latitudes

Bollgard<sup>TM</sup> lines have no specific added genes for cold tolerance or winter hibernation; no overwintered plants are possible. No cultivation of these cotton lines in any location in Canada will occur.

The biology of *Gossypium hirsutum*, described in the submission, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by Monsanto, Bollgard<sup>TM</sup> lines were determined not to be different from their counterparts in this respect.

## **2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive**

The pollen is heavy and sticky and cross pollination usually occurs in the presence of insect pollinators (bumble bees and honey bees). Cotton is normally self pollinating and the range of natural crossing among cotton plants is very limited.

Wild species of cotton (*G. barbadense* and *G. tomentosum*) occur only in arid parts of the tropics and sub-tropics. There are no wild relatives in Canada that can naturally hybridize with *G. hirsutum*. AAFC therefore concludes that gene flow from Bollgard<sup>TM</sup> lines to cotton relatives is not possible in Canada.

## **3. Altered Plant Pest Potential**

The intended effects of both novel traits are unrelated to plant pest potential, and cotton is not a plant pest in Canada. In addition, agronomic characteristics of Bollgard<sup>TM</sup> lines, were shown to be within the range of values displayed by currently commercialized cotton varieties. Susceptibilities to diseases such as: bacterial blight; boll rot; Fusarium; Phymatotricum root rot; and verticillium wilt were unchanged, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that Bollgard<sup>TM</sup> lines do not display any altered plant pest potential.

## **4. Potential Impact on Non-Target Organisms**

Of the ten species of insect assayed, only the four Lepidopteran species were affected by the microbial *B.t.k.*-HD73 (corn earworm, tobacco budworm, tobacco hornworm and European cornborer). The test system included six non-target species representing the orders Coleoptera, Diptera, Orthoptera and Homoptera (boll weevil, Southern corn rootworm, Colorado potato beetle, yellow fever mosquito, German cockroach and green peach aphid). Dietary toxicity studies were performed using the microbial protein on beneficial insects (honeybee, ladybird beetle, green lacewing larvae and parasitic wasp.) No effect was observed on non-target insects at concentrations more than 100 fold higher than that used to control target insects.

Acute oral and short term studies were undertaken with albino mice and bobwhite quail. After 8 days there were no observable effects on mice fed the insecticidal protein at the highest dose of 4,300mg/kg body wt. Raw cottonseed was fed at up to 100,000 ppm to bobwhite quail with no observed effects. A compendium of studies published by the USEPA (1988) has shown that large doses of the *B.t.k.* protein either single or multiple doses and fed for up to 2 years had no adverse effect on selected laboratory animals. These observations were expected, as the novel proteins are rapidly inactivated in simulated mammalian stomach fluids by enzymatic degradation and pH-mediated proteolysis. The proteins expressed in Bollgard<sup>TM</sup> cotton were shown to be equivalent to the original microbial proteins produced by the common soil *B.t.k.* bacteria.

Based on the above, AAFC has determined that the use of Bollgard<sup>TM</sup> cotton lines, when compared with currently commercialized cotton varieties, will not result in altered impacts on interacting organisms, including humans, with the exception of sensitive lepidopteran caterpillars.

## 5. Potential Impact on Biodiversity

Bollgard<sup>TM</sup> lines have no novel phenotypic characteristics which would extend their use beyond the current geographic range of cotton production into Canada. Since cotton does not outcross to wild relatives in Canada, there will be no transfer of novel traits to unmanaged environments. AAFC has determined that the Bollgard<sup>TM</sup> cotton lines will have no impact on biodiversity.

## 6. Potential for Development of Lepidopteran Resistance to the IRC Cotton

Since none of the Bollgard<sup>TM</sup> cotton lines will be grown in Canada, the potential development of field resistance need not be considered. A small possibility exists that lepidopteran caterpillars feeding on stored cottonseed may develop resistance to the insecticidal protein, however none of these insects are pests of cultivated crops, nor are they normally controlled with *B.t.* pesticides.

## V. Nutritional Assessment Criteria as Livestock Feed

### 1. Anti-Nutritional Factors

Cottonseed contains a high number of anti-nutritional factors and raw seed is unsuitable for monogastric animals. Processing serves to lower the levels of endogenous toxins and renders the cottonseed meal suitable for livestock feed. The levels of gossypol, anthocyanin, flavonoid and tannin in the transformed lines were within acceptable level for cotton and within the range of the controls for the 3 lines. The levels of the cyclopropenoid fatty acids, malvalic, sterculic and dihydrosterculic acids were found to be within the normal range for cotton. The levels of aflatoxin were within the level of the controls for line 757 and 1076. Line 531 from the Arizona test site, had significantly lower associated aflatoxin, likely because of reduced feeding damage from the pink bollworm on the transformed plants. Cottonseed from this area and others where pink bollworm is a significant pest often is unsuitable for animal feed because of high levels of aflatoxin.

### 2. Nutritional Composition of the PNTs

Seed from the three PNT lines from four to six U.S. locations was shown to be substantially equivalent to the control Coker 312 line in concentration of protein, oil, carbohydrate and ash. Line 1076 contained slightly higher carbohydrate and slightly lower oil than the control line, but both were within the normal range for cottonseed. Some differences in fatty acid profile among varieties in some traits were observed, but these were inconsistent, and not attributed to the novel trait. Fatty acid concentration was within the normal published range for cottonseed. Additional analyses of composite samples of cottonseed products (raw meal, toasted meal, kernel, refined oil) from each line showed that the products from the PNT lines were similar in composition to the control line. A four week rat feeding trial showed no difference in weight gain of animals fed diets containing 10% raw cottonseed meal from line 531 vs C312.

Therefore, the introduction of the novel trait into *G. Hirsutum* resulting in Bollgard<sup>TM</sup> cotton would not likely result in any secondary effects affecting the composition or nutritional quality of the cultivar.

## VI. Regulatory Decision

Based on the review of data and information submitted by the Monsanto Company, and thorough comparisons of the Bollgard<sup>TM</sup> cotton lines with unmodified cotton counterparts, the Feed Section of the Plant Products Division has concluded that the novel genes and their corresponding traits do not in themselves raise any concerns regarding the safety or nutritional composition of the Bollgard<sup>TM</sup> lines. Cottonseed and cottonseed meal are currently listed in Schedule IV of the Feeds Regulations and are, therefore, approved for use in livestock feeds in Canada. As Bollgard<sup>TM</sup> cotton has been assessed and found to be substantially equivalent to traditional cotton varieties, with respect to safety and nutritional quality, Bollgard<sup>TM</sup> lines and their products are considered to meet the present ingredient definitions and are approved for use as livestock feed ingredients in Canada. None of the Bollgard<sup>TM</sup> cotton lines will be grown in Canada nor can the seed overwinter, therefore the release of the feed (meal or seed) into the environment would have no intended or unintended effect.

**Feed use, of the Bollgard<sup>TM</sup> cotton lines 531, 757 and 1076 and other lines derived from them, but without the introduction of any other novel trait, is therefore authorized.**

This bulletin is published by the Plant Health and Production Division, Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office at:

Plant Health and Production Division  
Canadian Food Inspection Agency  
59 Camelot Drive  
Nepean, Ontario, K1A 0Y9  
Tel: (613) 225-2342  
Fax: (613) 228-6629

---

Date Modified: 2001-07-17

  
[Top of Page](#)

[Important Notices](#)