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# Decision Document 2018-123 - Determination of the Safety of BASF Canada Inc. (*Gossypium hirsutum*) Event GHB811

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This Decision Document has been prepared to explain the regulatory decisions reached under Section 2.6 - [Guidelines for the Assessment of Novel Feeds: Plant Sources](#), of Chapter 2 of the RG-1 Regulatory Guidance: Feed Registration Procedures and Labelling Standards, and based on the environmental criteria in Directive 94-08 - [Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits](#).

The Canadian Food Inspection Agency (CFIA), specifically the Animal Feed Division of the Animal Health Directorate, with input from the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate, CFIA, has evaluated information submitted by BASF Canada Inc. This information is in regard to double herbicide tolerant cotton event GHB811. The CFIA has determined that feed derived from these modified plants does not present a significant risk to the environment, nor does it present livestock feed safety concerns when compared to currently commercialized cotton varieties in Canada.

Taking into account this evaluation, use as livestock feed of cotton event GHB811 is, therefore, authorized by the Animal Feed Division of the Animal Health Directorate as of October 19th, 2018. Any cotton lines derived from cotton event GHB811 may also be used as livestock feed, provided that

- i. no inter-specific crosses are performed
- ii. the intended uses are similar
- iii. it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently commercialized cotton and permitted to be used as livestock feed in Canada, in terms of their potential environmental impact and livestock feed safety and nutrition
- iv. the novel genes are expressed at a level similar to that of the authorized line

Cotton event GHB811 is subject to the same phytosanitary import requirements as the unmodified cotton varieties, and is required to meet the requirements of other Canadian legislation including, but not limited to, the *Food and Drugs Act* and the *Pest Control Products Act*.

Please note that the livestock feed safety of novel feeds is a critical step in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety by Health Canada, have been addressed separately from this review.

**October 19, 2018**

This bulletin was created by the Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office or the Animal Feed Division by visiting the [contact page](#).

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## I (1). Brief identification of the modified plant

**Designation of the Modified Plant:** Cotton Event GHB811, OECD Unique Identifier BCS-GH811-4

**Applicant:** BASF Canada Inc.

**Plant Species:** Cotton (*Gossypium hirsutum*)

**Novel Traits:** Herbicide-tolerant to glyphosate and to HPPD inhibitors

**Trait Introduction Method:** *Agrobacterium*-mediated transformation

**Intended Use of the Modified Plant:** Production of cotton for fibre, whole or crushed cottonseed and cottonseed meal (cake, grain, flakes, pellets) or roughage 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

BASF Canada Inc. has developed a cotton event that is tolerant to the herbicides glyphosate and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors. Cotton event GHB811 was developed by BASF Canada Inc. using recombinant deoxyribonucleic acid (rDNA) technology, resulting in the introduction of the 2mepsps gene derived from *Zea mays* and the hppdPfw336-1Pa gene derived from *Pseudomonas fluorescens*. These genes enable the expression of 2mEPSPS and HPPD W336 enzymes, which have a decreased binding affinity to the herbicides thus conferring tolerance to glyphosate and to HPPD inhibitors respectively.

BASF Canada Inc. has provided information on the identity of cotton event GHB811, a detailed description of the transformation method and information on the insert copy number and intactness, levels of proteins expression in the plant, and the role of the inserted sequences. The novel proteins were identified and characterized. Information was provided for the evaluation of potential toxicity of the novel proteins to livestock and non-target organisms and potential allergenicity of the novel proteins to humans and to livestock.

Cotton event GHB811 was field tested at eight sites in the United States in the 2014 and 2015 growing seasons. An unmodified control cotton variety (Coker 312) that shares the same genetic background as cotton event GHB811 was included in the trials to act as a comparator for cotton event GHB811. Seven reference cotton varieties were also included in the field trials to establish a typical cotton behavior reference range for phenotypic parameters.

Agronomic characteristics of cotton event GHB811, such as early stand counts, days and heat units to first flower, final stand count, plant height, total bolls, seed cotton yield, seed index, abiotic stressors, disease damage, etc., were compared to those unmodified control cotton variety and to the range established by the reference cotton varieties.

Nutritional components of cotton event GHB811 seed and forage, such as protein, fat, moisture, ash, fibre, amino acids, fatty acids, alpha tocopherol, minerals and anti-nutrients were compared to those of the unmodified control cotton variety and to the reference range established by the reference cotton varieties.

The Animal Feed Division of the Animal Health Directorate, CFIA, with input from the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate, CFIA, has reviewed the above information. The following assessment criteria, as described in Section 2.6 [Guidelines for the Assessment of Novel Feeds: Plant Sources](#), of Chapter 2 of the RG-1 Regulatory Guidance: Feed Registration Procedures and Labelling Standards and Directive 94-08 [Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits](#) were used to determine the safety and efficacy as livestock feed and the environmental safety of this novel feed:

- the potential impact of cotton event GHB811 on livestock nutrition
- the potential impact of cotton event GHB811 on animal health and human safety as it relates to the potential transfer of residues into foods of animal origin and worker/bystander exposure to the feed
- the potential for cotton event GHB811 to become a weed of agriculture or be invasive of natural habitats
- the potential for gene flow from cotton event GHB811 to sexually compatible plants whose hybrid offspring may become more weedy or more invasive
- the potential for cotton event GHB811 to become a plant pest
- the potential impact of cotton event GHB811 and its gene products on non-target organisms, including humans
- the potential impact of cotton event GHB811 on biodiversity

The Animal Feed Division has also considered whether feeds derived from cotton event GHB811 meet the definitions and requirements of feeds as listed in Schedule IV of the *Feeds Regulations*.

### III. Description of the novel traits

#### 1. Development method

Cotton event GHB811 was developed through *Agrobacterium*-mediated transformation of cells from cotton variety Coker 312 using a vector containing hppdPFW336-1Pa and 2mepsps expression cassettes, the spectinomycin selectable marker aaDA and other genetic elements. Transformed cells were selected on the basis of tolerance to tembotrione (HPPD inhibitor herbicide) and regenerated to produce plants. Surviving plants expressing HPPD

W336 were then self-pollinated to consequently be selected on the basis of tolerance to glyphosate. Cotton event GHB811 was identified as a successful transformant based on molecular analyses, herbicide tolerance efficacy and agronomic evaluations.

## 2. Tolerance to glyphosate

The herbicide glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in plants which is part of the shikimic acid pathway essential for the production of the aromatic amino acids. The inhibition of EPSPS ultimately leads to growth suppression or death of the plant. Cotton event GHB811 produces an EPSPS double mutant (2mEPSPS) enzyme which is driven by a constitutive promoter. The modified enzyme contains two amino acid substitutions that confer a decreased binding affinity for glyphosate compared to the wild type maize enzyme, imparting field level tolerance to glyphosate herbicides.

The 2mEPSPS protein expressed in cotton event GHB811 is the same as the 2mEPSPS protein expressed in other glyphosate tolerant crops that have been already authorized for livestock feed use in Canada, including cotton event GHB614 (DD2008-72) and soybean event FG72 (DD2012-91).

Samples of cotton tissues were obtained from plants grown at 3 field trials in the United States during 2015. Tissues were collected from unsprayed plants and plants sprayed with glyphosate. The expression levels of 2mEPSPS protein were evaluated by ELISA. The level of the 2mEPSPS protein in GHB811 cotton leaf, root, square, boll, whole plant and fuzzy seed ranged from 76.36 to 1762.54 µg/g dry weight basis (DW). The level of 2mEPSPS protein in pollen from GHB811 ranged from 12.86 to 33.47 µg/g fresh weight basis (FW). Very similar 2mEPSPS protein levels were observed in the tissues of herbicide-treated plants and not-treated plants.

To perform the safety assessment of the modified proteins for livestock use, it was necessary to express the 2mEPSPS protein in *Escherichia coli* (*E. coli*) production system to obtain sufficient quantities of the proteins. Equivalency between the cotton event GHB811-produced and the *E. coli*-produced HPPD W336 proteins was demonstrated by comparing their apparent molecular weights, immunoreactivity, glycosylation, N-terminal sequence analysis, intact molecular mass (UPLC-UV-MSE -Ultra-Performance Liquid Chromatography Ultraviolet Mass Spectrometry) and functional activity. This equivalence allowed the use of the *E. coli*-produced 2mEPSPS protein in studies to confirm the safety of the cotton event GHB811.

The potential allergenicity and toxicity of the 2mEPSPS protein to livestock and non-target organisms were evaluated. The Weight of evidence indicates that the 2mEPSPS protein is unlikely to be allergenic and toxic, based on the following information. The source of the 2mepsps gene, *Zea mays*, is not commonly associated with allergenicity and the 2mEPSPS protein amino acid sequence lacks relevant similarities to known allergens. Unlike many allergens, the 2mEPSPS protein is expressed at low levels in cotton event GHB811. The *E. coli*-produced 2mEPSPS protein was shown experimentally to be rapidly degraded in simulated gastric fluid and not to be heat stable. Finally, unlike many allergens, 2mEPSPS protein expressed in cotton event GHB811 was shown experimentally to be unglycosylated.

It was also concluded that the 2mEPSPS protein is unlikely to be toxic to livestock and non-target organisms because it lacks a mode of action that is intrinsically toxic to livestock or non-target organisms. Furthermore, the 2mEPSPS protein amino acid sequence lacks relevant similarities to known toxins and no adverse effects were observed when the microbial 2mEPSPS protein was ingested by mice at doses of approximately 2000 mg/ kg bwt.

For a more detailed discussion of the potential allergenicity and toxicity of the 2mEPSPS protein, see [Section V, part 2. Potential Impact of Cotton Event GHB811 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.](#)

## 3. Tolerance to isoxaflutole (IFT) and HPPD inhibitors

HPPD-inhibitor herbicides such as IFT, target the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme which is involved in the tyrosine catabolism. The IFT upon absorption by a plant is converted into the active form of the herbicide, diketonitrile (DKN). The DKN inhibits the HPPD enzyme resulting in the disruption of the biosynthesis of carotenoids and consequently leads to bleaching and death in susceptible plants. Cotton event GHB811 produces a HPPD mutant (HPPD W336) enzyme which is driven by a constitutive promoter. The modified HPPD W336 enzyme contains a single amino acid change, compared to the wild type *Pseudomonas fluorescens* HPPD enzyme. This modification results in a reduced interaction between the HPPD inhibitor IFT and the HPPD W336 enzyme, conferring an increased tolerance to the herbicide.

The HPPD W336 protein expressed in cotton event GHB811 is the same as the HPPD W336 protein expressed in soybean event FG72 (DD2012-91) previously authorized for livestock feed use in Canada.

Samples of cotton tissues were obtained from plants grown at 3 field trials in the United States during 2015. Tissues were collected from unsprayed plants and plants sprayed with IFT. The expression levels of HPPD W336 protein were evaluated by ELISA. The level of the HPPD W336 protein in leaf, root, square, boll, whole plant and fuzzy seed of the cotton event GHB811 ranged from 10.91 to 1673.89 µg/g DW. The level of the HPPD W336 protein in pollen from GHB811 ranged from below the lower limit of quantitation to 0.69 µg/g FW. Very similar HPPD W336 protein levels were observed in the tissues of herbicide-treated plants and not-treated plants.

To perform the safety assessment of the modified proteins for livestock use, it was necessary to express the HPPD W336 protein in *Escherichia coli* (*E. coli*) production system to obtain sufficient quantities of the proteins. Equivalency between the cotton event GHB811-produced and the *E. coli*-produced HPPD W336 proteins was demonstrated by comparing their apparent molecular weights, immunoreactivity, glycosylation, N-terminal sequence analysis, intact molecular mass (UPLC-UV-MSE -Ultra-Performance Liquid Chromatography Ultraviolet Mass Spectrometry) and functional activity. This equivalence allowed the use of the *E. coli*-produced HPPD W336 protein in studies to confirm the safety of the cotton event GHB811.

The potential allergenicity and toxicity of the HPPD W336 protein to livestock and non-target organisms were evaluated. The weight of evidence indicates that the HPPD W336 protein is unlikely to be allergenic and toxic, based on the following information. The source of the hppd gene, *Pseudomonas fluorescens* is not commonly associated with allergenicity or toxicity and the HPPD W336 protein amino acid sequence lacks relevant similarities to known allergens or toxins. Unlike many allergens, the *E. coli*-produced HPPD W336 protein was shown experimentally to be rapidly degraded in simulated gastric fluid and not to be heat stable. Finally, unlike many allergens, cotton event GHB811 HPPD W336 protein was shown experimentally to be unglycosylated.

It was also concluded that the HPPD W336 protein is unlikely to be toxic to livestock and non-target organisms because it lacks a mode of action to suggest that it is intrinsically toxic to livestock or non-target organisms. Furthermore, the HPPD W336 protein amino acid sequence lacks relevant similarities to known toxins and no adverse effects were observed when microbial HPPD W336 protein was ingested by mice at doses of approximately 2000 mg/ kg bwt.

For a more detailed discussion of the potential allergenicity and toxicity of the HPPD W336 protein, see [Section V, part 2. Potential Impact of Cotton Event GHB811 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.](#)

#### 4. Stable integration into the plant genome

Molecular characterization by Southern blot analysis and PCR amplification and sequencing demonstrated that cotton event GHB811 contains one intact copy of the gene cassette containing the 2mepsps and the hppdPw336 genes and their regulatory elements inserted at a single site in the cotton genome. No additional elements,

including intact or partial DNA fragments of the gene cassette or backbone sequences from the plasmid vector, linked or unlinked to the intact insert were detected in cotton event GHB811.

The stability of the insert was verified by Southern blot analysis across five generations in the breeding history of cotton event GHB811. The inheritance pattern of the insert was evaluated by Real-Time PCR across three segregating generations of cotton event GHB811. The combined analysis showed that the insert is stably inherited and segregates according to the Mendelian rules of inheritance for a single genetic locus.

## **IV (4). Criteria for the environmental assessment**

Lines derived from cotton event GHB811 will not be grown in Canada. However, Canada imports cottonseed, as well as a wide range of other cotton products, that are used as human food, livestock feed or other industrial products.

### **1. Potential for cotton event GHB811 to become a weed of agriculture or be invasive of natural habitats**

Cotton (*Gossypium hirsutum*) is a member of the family *Malvaceae*. It is a perennial species cultivated as an annual and grown in the U.S., mostly in areas from Virginia southward and westward to California. Cotton is not grown in Canada as it is not adapted to environmental conditions found at these latitudes.

Cotton is not considered a weed in the regions where it is grown, nor is it invasive of unmanaged habitats in Canada. Cotton event GHB811 has not been modified to have altered cold-tolerance, and information supplied by BASF Canada Inc. indicates that the reproductive and survival biology of cotton event GHB811 is unchanged compared to its unmodified control.

The CFIA has therefore concluded that cotton event GHB811 is unlikely to become a weed of agriculture or invasive of natural habitats in Canada.

### **2. Potential for gene flow from cotton event GHB811 to sexually compatible plants whose hybrid offspring may become more weedy or more invasive**

Cotton is predominately self-pollinated. Although cross-pollination may occur at low levels, particularly in the presence of pollinators such as honeybees, cotton has no sexually compatible relatives native to Canada. Sexually compatible relatives of commercial cotton, such as *Gossypium barbadense* and *G. tomentosum*, are found only in tropical and sub-tropical regions.

This information, together with the fact that the novel traits have no intended effects on cotton reproductive biology, led the CFIA to conclude that gene flow from cotton event GHB811 to sexually compatible relatives in Canada is not possible.

### **3. Altered plant pest potential of cotton event GHB811**

Cotton is not a plant pest in Canada, and the intended effect of the novel trait is unrelated to plant pest potential (i.e., the potential for the plant to harbor new or increased populations of pathogens or pests). In addition, agronomic characteristics of cotton event GHB811 are similar to those described for currently commercialized cotton varieties.

The CFIA has therefore determined that cotton event GHB811 does not present a plant pest concern.

## 4. Potential impact of cotton event GHB811 on non-target organisms

The herbicide tolerance traits introduced into cotton event GHB811 are unrelated to a potential impact on non-target organisms. The hppdPfw336 and 2mEPSPS expression cassettes introduced into cotton event GHB811 are the same as those introduced into soybean event FG72 (DD2012-91) which has been previously authorized for unconfined environmental release in Canada.

Detailed characterization of the HPPD W336 and 2mEPSPS proteins expressed in cotton event GHB811 led to the conclusion that none of these proteins displays any characteristic of a potential toxin or allergen (see [III. Description of the Novel Traits](#)). Therefore, no negative impacts resulting from exposure of organisms to the HPPD W336 and 2mEPSPS proteins expressed in cotton event GHB811 are expected.

Composition analyses showed that the levels of key nutrients and anti-nutrients in seed from cotton event GHB811 are comparable to those in conventional cotton varieties (see [Section V, part 1. Potential Impact of Cotton Event GHB811 on Livestock Nutrition](#)). Therefore, it is very unlikely that the introduction of the novel traits may have caused unintended changes to the composition of cotton event GHB811 that would negatively impact organisms interacting with cotton event GHB811.

Cotton event GHB811 will not be grown in Canada. In the event that seed from cotton event GHB811 is accidentally released into the environment, any resulting plants would not be expected to set seed. Therefore, exposure of non-target organisms to the HPPD W336 and 2mEPSPS proteins from cotton event GHB811 is expected to be minimal to non-existent.

Based on the above, the CFIA has determined that the use of cotton event GHB811 will not result in altered impacts on interacting organisms in Canada, including humans, when compared to currently commercialized cotton varieties.

## 5. Potential impact of cotton event GHB811 on biodiversity

No varieties of cotton, nor any sexually compatible relatives that can readily interbreed with cotton, can grow in the Canadian environment since cotton is not adapted to the environmental conditions encountered in Canadian agricultural environments. In addition, cotton event GHB811 has no observed or expected modifications that would allow it to survive in the Canadian environment and, as a result, is not expected to enter or survive in managed or unmanaged ecosystems in Canada. Furthermore, there is no evidence to suggest that cotton event GHB811 would cause negative impacts on interacting organisms.

The CFIA has therefore concluded that cotton event GHB811 does not present any adverse impacts on biodiversity in Canada.

## V (5). Criteria for the livestock feed assessment

The Animal Feed Division considered the nutrient and anti-nutrient profiles of the cotton event GHB811. It also took into consideration the safety of feed ingredients derived from it, including the presence of gene products, residues and metabolites in terms of animal health and human safety as it relates to the potential transfer of residues into foods of animal origin and worker/bystander exposure to the feed. Finally, the Animal Feed Division sought to determine whether feeds derived from the cotton event GHB811 meet the definitions and requirements of feeds as listed in Schedule IV of the *Feeds Regulations*.

### 1. Potential impact of cotton event GHB811 on livestock nutrition

#### Nutritional and anti-nutrient composition

The nutritional equivalence between cotton event GHB811 (unsprayed and sprayed with glyphosate and isoxaflutole), the unmodified control cotton variety (Coker 312) and seven reference cotton varieties was determined from eight replicated field trials in the United States during the 2014 and 2015 growing seasons. Cottonseed samples collected at maturity from eight United States field trial sites, were ginned to produce fuzzy seed which were analyzed for proximate, fiber, amino acids, fatty acids, minerals, alpha tocopherol, and anti-nutrients (Free and total gossypol; dihydrosterulic acid, sterulic acid and malvalic acid) as recommended by the OECD consensus document for new varieties of cotton ([OECD, 2009 \(PDF 328 kb\)](#)). Samples from one non replicated site trial in Argentina was processed to fuzzy seed, delinted seed, linters, hulls, toasted meal, untoasted meal, crude oil and deodorized (RBD) oil and analyzed for proximate, minerals, tocopherols, anti-nutrients, amino acids and fatty acids in various components as well.

Composition data was analyzed statistically using analysis of variance and statistical differences observed between cotton event GHB811 and the unmodified control cotton variety were identified ( $P < 0.05$ ). The biological relevance of any significant difference between cotton event GHB811 and the unmodified control cotton variety was assessed within the range of the values obtained from the reference control cotton varieties and in the published scientific literature and/or the ILSI Crop Composition Database ([ILSI-CCDB](#)). Mean and standard deviation were computed for the data generated from the Argentina field trial. Statistical comparison between events was therefore not possible.

There were no statistically significant differences between the unmodified control cotton variety and cotton event GHB811 (sprayed and unsprayed) fuzzy seeds for crude fat, ash, acid detergent fibre (ADF), total dietary fibre (TDF) and carbohydrates (calculated).

Crude protein was significantly lower in cotton event GHB811 (sprayed and unsprayed) compared to the unmodified control cotton variety; however the means were within the range of the reference cotton varieties grown in the US trials. Except for cysteine, methionine, in cotton event GHB811 (sprayed) and palmitoleic and stearic acids (GHB811 (sprayed and unsprayed)). No significant differences were observed between the unmodified control cotton variety and cotton event GHB811 (sprayed and unsprayed) for all other amino acids and fatty acids. Cysteine and methionine levels in cotton event GHB811 (sprayed) and palmitoleic and stearic acid in GHB811 (sprayed and unsprayed) were however within the range of the reference cotton varieties, and therefore the differences were not considered to be biologically relevant. No significant difference were observed between the unmodified control cotton variety and cotton event GHB811 (sprayed and unsprayed) for all mineral analyzed.

Although statistically significant differences were noted for alpha tocopherol, dihydrosterulic acid, free and total gossypol total tocopherols between fuzzy seeds cotton event GHB811 and the unmodified control cotton variety, the mean values were within the range of the values observed in the reference control cotton varieties grown in the trial; and therefore the differences were not considered biologically relevant. No significant differences were observed between the unmodified control cotton variety and cotton event GHB811 (sprayed and unsprayed) for sterulic and malvalic acids.

## Conclusion

It was concluded based on the evidence provided by BASF Canada Inc. that the nutritional composition of cotton event GHB811 is similar to that of the unmodified control cotton varieties.

## **2. Potential impact of cotton event GHB811 on animal health and human safety as it relates to the potential transfer of residues into foods of animal origin and worker/bystander exposure to the feed**

The cotton event GHB811 is tolerant to glyphosate and HPPD inhibitors as a result of insertion of genes encoding 2mEPSPS and HPPD W336 proteins, respectively. The assessment of cotton event GHB811 evaluated the impact of the following potential hazards relative to the safety of feed ingredients derived from this event: The presence of the proteins 2mEPSPS and HPPD W336.

## 2mEPSPS protein

To obtain sufficient quantities of 2mEPSPS protein for the assessment of feed safety, it was necessary to produce the 2mEPSPS protein in an *Escherichia coli* (*E. coli*) expression system. The GHB811 cotton-produced and the *E. coli*-produced 2mEPSPS proteins were demonstrated to be equivalent by comparing their molecular weight, immunoreactivity, and functional activity. This allowed the *E. coli*-produced 2mEPSPS protein to be used in studies to confirm the safety of the 2mEPSPS protein in cotton event GHB811.

The potential allergenicity of the 2mEPSPS protein to livestock was evaluated. With respect to its potential allergenicity, no single experimental method yields decisive evidence, thus a weight-of-evidence approach was taken, taking into account information obtained with various test methods. The source of the gene encoding the 2mEPSPS protein, *Zea mays*, is not known to produce allergens and a bioinformatics evaluation of the 2mEPSPS protein amino acid sequence confirmed the lack of relevant similarities between the 2mEPSPS protein and known allergens. *E. coli*-produced 2mEPSPS protein safety studies indicated that, unlike many allergens, this protein is rapidly degraded in simulated gastric fluid and is not heat stable. Finally, unlike many allergens, cotton event GHB811 2mEPSPS protein was shown experimentally to be unglycosylated. The weight of evidence indicates that the 2mEPSPS protein is unlikely to be allergenic.

In terms of its potential toxicity to livestock, the 2mEPSPS protein lacks a mode of action to suggest that it is intrinsically toxic to livestock and a bioinformatics evaluation of the 2mEPSPS protein amino acid sequence confirmed the lack of relevant similarities between the 2mEPSPS protein and known toxins. In addition, acute, *E. coli*-produced 2mEPSPS protein, safety studies indicated that no adverse effects were observed when the 2mEPSPS protein was ingested by mice at doses of approximately 2000 mg/kg bw. This information indicates that the 2mEPSPS protein is unlikely to be toxic to livestock.

The livestock exposure to the 2mEPSPS protein is expected to be negligible, as the 2mEPSPS protein is expressed at very low levels in cotton event GHB811 and is rapidly degraded under conditions which simulate the mammalian digestive tract.

## HPPD W336 protein

To obtain sufficient quantities of HPPD W336 protein for the assessment of feed safety, it was necessary to produce HPPD W336 protein in an *E. coli* expression system. The GHB811-cotton produced and the *E. coli*-produced HPPD W336 proteins were demonstrated to be equivalent by comparing their molecular weight, immunoreactivity, and functional activity. This allowed the *E. coli*-produced HPPD W336 to be used in studies to confirm the safety of the HPPD W336 protein in cotton event GHB811.

The potential allergenicity of the HPPD W336 protein to livestock was evaluated. With respect to its potential allergenicity, no single experimental method yields decisive evidence, thus a Weight of evidence approach was taken, taking into account information obtained with various test methods. The source of the gene encoding the HPPD W336 protein, *Pseudomonas fluorescens*, is not known to produce allergens and a bioinformatics evaluation of the HPPD W336 protein amino acid sequence confirmed the lack of relevant similarities between the HPPD W336 protein and known allergens. *E. coli*-produced HPPD W336 protein safety studies indicated that the protein was heat stable. However, unlike many allergens, the *E. coli*-produced protein was found to be rapidly degraded in simulated gastric fluid and the cotton event GHB811-produced HPPD W336 protein was shown experimentally to be unglycosylated. The weight of evidence indicates that the HPPD W336 protein is unlikely to be allergenic.

In terms of its potential toxicity to livestock, the HPPD W336 protein lacks a mode of action to suggest that it is intrinsically toxic to livestock and a bioinformatics evaluation of the HPPD W336 protein amino acid sequence confirmed the lack of relevant similarities between the HPPD W336 protein and known toxins. In addition, acute *E. coli*-produced HPPD W336 protein safety studies indicated that no adverse effects were observed when the HPPD W336S protein was ingested by mice at doses of approximately 2000 mg/kg bw. This information indicates that the HPPD W336 protein is unlikely to be toxic to livestock.

The livestock exposure to the HPPD W336 protein is expected to be negligible as the HPPD W336 protein is expressed at very low levels in cotton event GHB811 and is rapidly degraded under conditions which simulate the mammalian digestive tract.

## Conclusion

It was concluded, based on the evidence provided by BASF Canada Inc., that the novel 2mEPSPS and HPPD W336 proteins which confer tolerance to glyphosate herbicides and HPPD inhibitors, respectively, will not confer to cotton event GHB811 any characteristic that would raise concerns regarding the safety of cotton event GHB811. Feed ingredient(s) derived from cotton event GHB811 are considered to meet present ingredient definitions for cotton in the *Feeds Regulations* and as such are approved for use as livestock feed in Canada.

## VI. New information requirements

If, at any time, BASF Canada Inc. becomes aware of any new information regarding risk to the environment, livestock or human health that could result from the release or livestock feed use of cotton event GHB811 or lines derived from it, BASF Canada Inc. is required to immediately provide such information to the CFIA. On the basis of such new information, the CFIA will re-evaluate the potential impact of cotton event GHB811 on the environment, livestock and human health and may re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of cotton event GHB811.

## VII. Regulatory decision

Cotton event GHB811 will not be grown in Canada nor can the seed overwinter; therefore, the release of the feed into the environment would result in neither intended nor unintended environmental effects.

Based on the review of the data and information submitted by BASF Canada Inc. and input from other relevant scientific sources, the Animal Feed Division has concluded that the novel 2mEPSPS and HPPD W336 protein-based herbicide tolerance traits will not confer to cotton event GHB811 any characteristic that would raise concerns regarding the safety or nutritional composition of cotton event GHB811. 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. Cotton event GHB811 has been found to be as safe and nutritious as currently and historically grown cotton varieties. Cotton event GHB811 and its products are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

Taking into account this evaluation, use as livestock feed of cotton event GHB811 is, therefore, authorized by the Animal Feed Division of the Animal Health Directorate as of October 19, 2018. Any cotton lines derived from cotton event GHB811 may also be used as livestock feed, provided that:

- i. no inter-specific crosses are performed
- ii. the intended uses are similar
- iii. it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently commercialized cotton and permitted to be used as livestock feed in

Canada, in terms of their potential environmental impact and livestock feed safety

iv. the novel genes are expressed at a level similar to that of the authorized line

Cotton event GHB811 is subject to the same phytosanitary import requirements as the unmodified cotton varieties, and is required to meet the requirements of other Canadian legislation including, but not limited to, the *Food and Drugs Act* and the *Pest Control Products Act*.

Please refer to [Health Canada's Decisions on Novel Foods](#) for a description of the food safety assessment of cotton event GHB811.

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