Case Study: Cry1F Protein (TC1507)

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• Some insect resistance crops have been produced by genetic modification with genes from the gram-positive bacterium *Bacillus thuringiensis* (*Bt*) commonly found in soil.

• The insecticidal crystal Cry1F protein is derived from *Bt* and is effective at controlling lepidopteran pests in regard to maize.

• Insect resistant GM maize product that contains the *cry 1F* gene that encodes the Cry1F protein was grown commercially in the US for the first time in 2003.
Product Safety Assessment

- Allergenicity
- Toxicology (mammalian)
- Environmental
  - Gene Flow
  - Environmental Fate/Exposure
  - Ecotoxicology
- Insect Resistance Management

Human Dietary Exposure Assessment
# Substantial Equivalence: Nutrients

## Proximates and Fiber (7)
- Crude Protein
- Crude Fat
- Ash
- Crude Fiber
- Acid detergent fiber
- Neutral detergent fiber
- Carbohydrates

## Fatty Acids (25)
- Caprylic acid (C8:0)
- Capric acid (C10:0)
- Lauric acid (C12:0)
- Myristic acid (C14:0)
- Myristoleic acid (C14:1)
- Pentadecanoic acid (C15:0)
- Pentadecenoic acid (C15:1)
- Palmitic acid (C16:0)
- Palmitoleic acid (C16:1)
- Heptadecanoic acid (C17:0)
- Heptadecenoic acid (C17:1)
- Stearic acid (C18:0)
- Oleic acid (C18:1)
- Linoleic acid (C18:2)
- (9,15) Isomer of linoleic acid (C18:2)
- Linolenic acid (C18:3)
- γ-linolenic acid (C18:3)
- Arachidic acid (C20:0)
- Eicosenoic acid (C20:1)
- Eicosadienoic acid (C20:2)
- Eicosatrienoic acid (C20:3)
- Arachidonic acid (C20:4)
- Behenic acid (C22:0)
- Erucic acid (C22:1)
- Lignoceric acid (C24:0)

## Vitamins (24)
- Vitamin B1
- Vitamin B2
- Pantothenic acid
- Vitamin B6
- Niacin
- Folic acid
- α-tocopherol
- β-tocopherol
- δ-tocopherol
- γ-tocopherol
- Total tocopherols
- Isoflavones
- Genistin
- Genistein
- Malonylgenistin
- Acetylgensitin
- Daidzin
- Daidzein
- Malonyldaidzin
- Acetylaidzin
- Glycitin
- Glycitein
- Malonylglycitin
- Acetylglucitlin

## Amino Acids (18)
- Methionine
- Cystine
- Lysine
- Tryptophan
- Thrreonine
- Isoleucine
- Histidin
- Valine
- Leucine
- Arginine
- Phenylalanine
- Glycine
- Alanine
- Aspartic acid
- Glutamic acid
- Proline
- Serine
- Tyrosine

## Minerals (9)
- Calcium
- Phosphorous
- Magnesium
- Manganese
- Copper
- Iron
- Potassium
- Sodium
- Zinc

## Oligosaccharides (4)
- Sucrose
- Stachyose
- Raffinose

## Secondary Metabolites & Anti-Nutrients (4)
- Coumestrol
- Lectins
- Phytic acid
- Trypsin Inhibitor
-Comparison of the GM crop to a conventional equivalent with a History of Safe Use (HOSU) guides the safety assessment
Protein Allergy Assessment
Codex guideline – Weight of Evidence
Protein Allergenicity Assessment

CODEX, 2009

- Glycosylation
- Immunological Methods**
- Heat Lability
- Stability to Pepsin
- Bioinformatics
- Gene source
- Expression Levels

**if necessary
Gene Source: *Bacillus thuringiensis* (Bt)

- Ubiquitously found throughout the environment in soil, on leaf surfaces, and aquatic environments.

- Used as a biopesticide on organic food products. In over 40 years of commercial use as biopesticides, *Bt* microbial preparations containing Cry proteins have been used safely with no adverse reports of human health or environmental effects (McClintock et al., 1995; Siegel, 2001).

- *Bt* bacteria are not an allergenic source. There are no clinical data to support any allergic reactions to *Bt*, including occupational allergy associated with the manufacture of products containing *Bt* (EPA, 2000, 2005).

- *Bt* derived proteins are not contained in any protein allergy databases (e.g., University of Nebraska) as there are no clinical data indicating they are allergens or cross-reactive with known protein allergens.
Allergen Search Strategy (Bioinformatics)

- Compare amino acid sequence of query protein to database containing sequences of food, dermal and respiratory allergens.

• Evaluate sequence for amino acid identity using local alignment programs, such as BLAST (or FASTA)
  • $\geq 35\%$ identity over an 80 or greater amino acid window

and potential (theoretical) IgE epitope matches.

Welcome to AllergenOnline.org

AllergenOnline provides access to a peer reviewed allergen list and sequence searchable database intended for the identification of proteins that may present a potential risk of allergenic cross-reactivity. This website was designed to help in assessing the safety of proteins that may be introduced into foods through genetic engineering or through food processing methods. The objective is to identify proteins that may require additional tests, such as serum IgE binding, basophil histamine release or in vivo challenge to evaluate potential cross-reactivity.

The database is updated annually. Version 4 was released on a public website in 2004. The database is freely accessible with the intent of providing a simple and useful tool that may be useful in food safety evaluations.

Features and Tools Available.

Sequence search routines for food safety
- We continue provide simple amino acid search routines to allow you to compare a protein sequence with the sequences in the current AllergenOnline database, which is updated on an annual basis. This is intended primarily for evaluating new proteins in Genetically Modified crops or in Novel Foods.
- Search for full-length alignments by FASTA: The most predictive search is the overall FASTA alignment (see FASTA Help Page), with identity matches greater than 50% indicating possible cross-reactivity (Aalberse, 2000).
- Search for 80 amino acid alignments by FASTA: A precautionary search using a sliding window of 80 amino acid segments of each protein to find identities greater than 35% (according to CODEX Alimentarius guidelines, 2003).
- Search for 8 amino acid exact match: An 8-amino acid short-sequence identity search is provided since some regulatory authorities demand results of this extremely precautionary search. Our scientific opinion
None of the proteins identified met or exceeded the threshold of greater than or equal to 35% identity over 80 or greater amino acid residues.

No stretches of eight or greater contiguous amino acids were shared between the Cry1F protein and proteins in the allergen database.

These data indicate the lack of both amino acid sequence identity and immunologically relevant similarities between the Cry1F protein and known or putative protein allergens.
Stability to Pepsin In Vitro

- Protein resistance to pepsin evaluated in simulated gastric fluid (pH 1.2) containing 0.3% (w/v) pepsin.

- Digestions performed for time intervals 0, 15 and 30 seconds, 1, 2, 5, 10, 15, 20, 30, and 60 minutes at 37°C.

- Samples (each protein at each time point) then analyzed by SDS polyacrylamide gel electrophoresis and/or Western blot analysis.

- A standardized protocol for evaluating the *in vitro* pepsin resistance of proteins was established (Thomas *et al.*, Regulatory Toxicology Pharmacology, 39:87-98, 2004).

Provides a loose correlation for major food allergens (stable).

This test is not meant to “mimic” real digestion.
The Cry1F protein hydrolyzed within one minute in pepsin

<table>
<thead>
<tr>
<th>Lane</th>
<th>Load Volume µL</th>
<th>Sample ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>Protein X (~2.3 µg) in water “Time 0”</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>SeeBlue molecular weight marker</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>Protein X (~2.3 µg) in SGF “Time 0”</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>Protein X in SGF for 0.5 minutes</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>Protein X in SGF for 1 minute</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>Protein X in SGF for 2 minutes</td>
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<tr>
<td>7</td>
<td>20</td>
<td>Protein X in SGF for 5 minutes</td>
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<td>Protein X in SGF for 10 minutes</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>Protein X in SGF for 20 minutes</td>
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<td>10</td>
<td>20</td>
<td>Protein X in SGF for 30 minutes</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>Protein X in SGF for 60 minutes</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>SGF control ~60 minutes</td>
</tr>
</tbody>
</table>
Heat Lability

- Cry1F protein is heat labile
- Cry1F protein loses immunoreactivity after heat processing and degrades quickly under pressures and heat commonly used in commercial processing of maize (Mayes, 1999).

Glycosylation

- The *in planta* derived Cry1F protein is not glycosylated
Taken together, these data indicate that the Cry1F protein is not a potential allergen.
Lack of cross-reactivity between the *Bacillus thuringiensis* derived protein Cry1F in maize grain and dust mite Der p7 protein with human sera positive for Der p7-IgE

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Abstract

Cry1F protein, derived from *Bacillus thuringiensis*, is effective at controlling lepidopteran pests and a synthetic Cry1F transgene was transferred into maize. For the safety assessment of genetically modified food crops, the allergenic potential of the introduced novel trait(s) is evaluated. Because no single parameter is currently predictive of allergic potential, a 'weight of evidence' approach has been proposed. As part of this assessment, the amino acid (aa) sequence of the Cry1F protein was compared to a database of known allergens using recommended criteria. The Cry1F protein did not show significant similarity or a match of eight contiguous identical aa with any allergen. However, a single six contiguous aa match was identified between Cry1F and the Der p7 protein of the dust mite, *Dermatophagoides pteronyssinus*. To investigate whether Cry1F was cross-reactive with Der p7, sera from 10 dust mite allergic patients containing Der p7-specific IgE antibody were used to compare IgE-specific binding. No evidence of cross-reactivity was observed between Cry1F and Der p7. This study provides in vitro IgE sera screening data, that when considered in the context of other bioinformatic data [Hileman R.E., Silvanovich, A., Goodman R.E., Rice E.A., Holleschak G., Astwood J.D., Hefle S.L., 2002. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. Int. Arch. Allergy Immunol. 128. 280–291: Stadler. M.B., Stadler. B.M. 2003. Allergenicity
Holistic approach

- Host plant
  - Inherent toxicity?
  - Inherent allergenicity?

- Gene
  - HOSU of source?
  - Insertion consequences?
  - Horizontal transfer?

- Gene product
  - Toxic?
  - Allergenic?

- GM Plant
  - Substantial Equivalence?
  - Increase of toxicity?
  - Increase of allergenicity?
Toxicology Safety Assessment
Weight of Evidence

- History of Safe Use
- Heat Lability
- Acute Toxicology study
- In Vitro Digestibility
- Mode of Action & Specificity
- Bioinformatics
- Expression Level & Dietary Intake
History of Safe Use in Food

- Used as a biopesticide on organic food products. In over 40 years of commercial use as biopesticides, *Bt* microbial preparations containing Cry proteins have been used safely with no adverse reports of human health or environmental effects (McClintock et al., 1995; Siegel, 2001).

- Crops containing *Bt* proteins as *in planta* insecticides have been grown since 1996 with no reports of adverse health effects.
Expression of the Cry1F protein confers *in planta* resistance to corn borer and other Lepidopteran species.

Toxicity to insects is attributable to interaction between *Bt* proteins and high affinity receptors in gut epithelial cells specific to target Lepidopteran species (*but not in non-target species including mammals*).

Leads to pore formation and lysis of gut cells which cause the insects to stop feeding and ultimately die.
Bioinformatic Analysis (Potential Protein Toxin Similarity)

• Bioinformatic analysis

  – BLASTP search against the Genpept dataset [millions of proteins] representing submitted protein sequences and translations of all submitted nucleotide sequences

  – Comparison of Cry1F protein sequence found no similarity to known protein toxins or anti-nutrients
- Pepsin digestion - < 1 min
- Heat labile
Why Acute Toxicology Studies?

- Protein toxins act through acute mechanisms after the administration of a single dose at doses in the nanogram to milligram per kilogram body weight.
- Therefore, acute oral toxicity studies using gram per kilogram body weight doses of the novel protein are appropriate for assessing the potential toxicity of proteins.
Acute Oral Toxicity Test \textit{(in vivo)}

- Details
  - Typically conducted in mice (5 male and/or 5 female)
  - Recombinant transgene protein (2-5 g)
    - Need to test protein representative of form expressed \textit{in planta} (biochemical equivalence)
  - One time oral gavage up to “limit” dose:
    - 2000 mg/kg (OECD) or 5000 mg/kg
    - Equivalent to 140 g of protein in average human
  - Evaluate (14 day observation):
    - Mortality, body weight, behavioral endpoints, gross necropsy
  - Conclusion:
    - Transgene protein is or is not acutely toxic
Cry1F Acute Toxicity

- 5050 mg/kg administered (11.4%)
- 576 mg/kg of Cry1F protein administered
- LD50 > 576 mg/kg
Cry1F Maize: Estimate of Acute Dietary Margin of Exposure

The estimated 95th percentile per capita exposure for acute dietary consumption of Cry1F protein in maize and maize products is 0.047 mg (kg BW)^{-1} for the most sensitive population (non-nursing infants, less than 1 year old).

This exposure estimate is based on the maximum measured level for Cry1F protein in maize grain (3.4 mg kg^{-1}, range 1.7 – 3.4 mg Cry1F kg^{-1}). (Very low expression levels - ppm range)

Estimate is very conservative in that it uses maximum anticipated residue levels in all corn and corn products, it considers 100% of crop treated (that is Cry1F protein occurrence in all corn and corn products), and it includes no factors to account for food processing.

The acute dose (576 mg/kg) represents a value 12,190 X greater than estimated 95th percentile dietary exposure and can be used as a conservative estimate of the margin of exposure.
Weight of Evidence Cry1F Protein

- Heat Lability (yes)
- History of Safe Use (yes)
- Acute Toxicology study (LD50 > 576 mg/kg)
- In Vitro Digestibility (< 1 min)
- Bioinformatics (no matches to protein toxins)
- Expression Level (low-ppm) & Dietary Intake (large margin of exposure)
- Mode of Action & Specificity (well-defined)
Holistic approach

- Unintended effects: predictable and unpredictable effects
- Part of plant consumed by humans (typically grain)
Cry1F Subchronic Study

• Subchronic rodent feeding study
  – A general but comprehensive health screen
  – Relatively long term exposure that includes development and maturation
    • 90 Days in duration (13 weeks); diet fed ad libitum (33 and 11% Cry1F maize)
    • Control is closest genetic non-GM comparator; non-GM Reference Standards (33 and 11%) (acceptable range of normal variation)
      • 12 animals/sex per test group
  – Goal is to determine if unintended differences occurred during production of a GM resulting in adverse effects
• Subchronic rodent feeding studies
  – Nutritional performance (in-life evaluations)
    • Body weight
    • Feed consumption
    • Feed efficiency
    • Detailed clinical observations
    • Neurobehavioral (motor activity; FOB)
    • Mortality
  – Extensive Clinical Pathology evaluation Following In-Life Phase
    • Hematology (16); Coagulation (2)
    • Organ Weights; Pathology (~40 organs)
    • Clinical Chemistry (18)
    • Urinalysis
Conclusions: TC1507 Maize Subchronic Study

- No toxicologically significant differences observed between any pair of treatment groups
  - Nutritional performance variables
  - Clinical and neurobehavioral signs
  - Ophthalmology
  - Clinical pathology
  - Organ weighs
  - Gross and microscopic pathology

- 1507 maize grain is as safe and nutritious as non-GM maize
Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague-Dawley rats

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Abstract

Maize line 1507, containing event DAS-Ø15Ø7-1 (1507), is a genetically modified (GM) maize plant that expresses the cry1F gene from Bacillus thuringiensis (Bt) subspecies aizawai and the phosphinothricin-N-acetyltransferase (pat) gene from Streptomyces viridochromogenes throughout the plant including in the grain expression of the Cry1F protein confers in planta resistance to the European corn borer (ECB; Ostrinia nubilalis Hübner: Crambidae) and other lepidopteran pests. Expression of the PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium. The current study evaluated the nutritional performance of rats fed diets containing 1507 maize grain in a subchronic rodent feeding study. The grains in this study, 1507, its near-isogenic control (33P66), and a non-GM
Substantial Equivalence: Feed Safety and Performance (Nutritional Equiv.)

Animals are fed GM crops and control conventionally bred crops in balanced diets: animal production and health is evaluated

- Feed Intake
- Body Weight
- Carcass Yield
- Feed Efficiency
- Feed Conversion
- Milk Yield
- Milk Composition
- Nutrient Composition
- Digestibility
Growth Performance and Carcass Composition of Pigs Fed Corn Grain from DAS-Ø15Ø7-1 (Herculex I) Hybrids

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ABSTRACT

The objective of this experiment was to evaluate a transgenic source of corn grain containing the event DAS-Ø15Ø7-1 were calculated. Hot carcass weights, back fat thickness, loin eye area, and loin eye depth were measured at slaughter, and dressing percentage and average lean meat percentage were calculated. Results widely adopted for commercial use in the United States since 1996. Insect-protected, herbicide-tolerant, and stacked gene varieties accounted for 73% of all corn planted in the United
Performance of Lactating Dairy Cows Fed Silage and Grain from a Maize Hybrid with the cry1F Trait Versus its Nonbiotech Counterpart

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ABSTRACT

Effects of feeding grain and maize silage from a non-Bt maize and a variety of Bt maize that contains cry1F (event TC1507, event DAS-Ø15Ø7-1), a gene that provides maize with insect resistance, on the health and performance of dairy cows were evaluated. In a crossover trial, 20 lactating Holstein cows were assigned to each of 2 dietary treatment groups and fed diets containing whole-plant maize silage plus maize grain from TC1507 or its near-isoline counterpart (control). There were no differences in any of these indices of health status. Further, hematological profiles for cows in the dietary treatment groups were not different. In summary, no differences were detected in milk production, milk composition, or cow health as indicated by physical measures, blood chemistry, and hematological analyses between dairy cows fed diets containing maize grain plus whole-plant maize silage from TC1507 and dairy cows fed grain plus silage from its near-isoline counterpart.

Key words: cry1F gene, maize grain, maize silage, dairy cow.
Evaluation of Nutritional Equivalency of Corn Grain from DAS-Ø15Ø7-1 (Herculex* I) in the Diets of Laying Hens

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Primary Audience: Researchers, Nutritionists

SUMMARY

Grain from transgenic corn line TC1507 (Herculex* I) from Pioneer Hi-Bred International Inc. and Dow AgroSciences LLC, which expresses the Cry1F protein from Bacillus thuringiensis to provide protection from lepidopteran pests of corn, was compared with its isoline equivalent and 2 conventional corn strains in a 16-wk laying hen feeding trial. Egg production and production efficiency of hens fed the diet formulated with transgenic grain TC1507 were similar to those of hens fed diets formulated with isoline or nontransgenic conventional corns. Hens fed TC1507 had similar egg qualities as those fed nontransgenic grain diets. Diet × phase interactions were noted for Haugh unit and Roche color fan score. Hens fed conventional corn
Broiler study nutritional evaluation of b.t. cry1f maize corn from bacillus thuringiensis subsp. aizawai and phosphinothricin-n-acetyltransferase. J. L. McNaughton*1 and L. Zeph2, 1Solution BioSciences, Inc., Salisbury, MD, 2Pioneer Hi-Bred International, Inc. Johnston, IA.

Two maize (corn) lines have been modified to express both the Cry1F protein from Bacillus thuringiensis subsp. aizawai and the phosphinothricin-N-acetyltransferase (PAT) protein, referred to as B.t. Cry1F maize lines 1507 or 1360 (Cry1F event TC1507 and event TC1360). Expression of Cry1F and PAT proteins provides control of European corn borer (ECB) and certain other lepidopteran pests, as well as conferring tolerance to glufosinate-ammonium herbicides. A study was conducted to determine the effect of diets containing maize from transgenic Cry1F hybrid maize lines on the performance of commercial broiler chickens (Cobb x Cobb strain) from 0-42 days of age when reared in wire floor cages. Seven reps, containing 5 male broiler chicks per rep, were fed diets containing either Cry1F event TC 1507 and/or event TC1360 and these maize sources were compared to four sources of U.S. corn commercial sources from various feed mill sources in the Eastern U.S. and a positive Control maize hybrid source 7250 (a non-transgenic control maize line). Maize (54.2% in starter and 57.0% in grower rations, across all treatments) - soybean type rations were employed throughout the study and fed ad libitum from 0-42 days of age. Prior to adding to rations, hybrid maize kernels from B.t. Cry1F event TC1507 or event TC1360, and control substances were analyzed for expression of the Cry1F protein using a specific ELISA. Cry1F proteins were confirmed to be present in the Cry1F event tC1507 and event TC1360 and absent in the control substances. Based on the results of this study, mortality, mean body weight, and feed conversion were statistically similar (P<0.05) among treatment groups. Therefore, maize grain from Cry1F event TC1507 and TC1360 are considered nutritionally equivalent to
Large Animal Feeding Studies

Conclusion

Maize grain from Cry1F event TC1507 was considered nutritionally equivalent to maize grain from commercial hybrids when fed to commercial broiler chickens, dairy cows, swine, laying hens, and cattle.
# Food and Feed Safety Assessment Studies for TC1507 Maize

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<thead>
<tr>
<th>Study Type</th>
<th>TC1507</th>
<th>Results</th>
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<tbody>
<tr>
<td>• Acute Oral Toxicity in Mice</td>
<td>✓</td>
<td>• No mortality noted with Cry1F pure protein</td>
</tr>
<tr>
<td>• Sub-chronic Feeding Study in Rats</td>
<td>✓</td>
<td>• TC1507 maize grain as safe and nutritious as non-transgenic grain</td>
</tr>
<tr>
<td>• Protein Thermal Stability</td>
<td>✓</td>
<td>• Cry1F protein not stable under heated conditions</td>
</tr>
<tr>
<td>• Pepsin Digestibility Assay</td>
<td>✓</td>
<td>• Cry1F protein readily digested by pepsin</td>
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<tr>
<td>• Livestock Feeding Study</td>
<td>✓</td>
<td>• TC1507 maize grain nutritionally equivalent to non-transgenic grains</td>
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<tr>
<td>• Compositional Analysis of Key Components</td>
<td>✓</td>
<td>• TC1507 maize substantially equivalent to non-transgenic maize</td>
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<tr>
<td>• Additional Studies</td>
<td>✓</td>
<td>• No homology of Cry1F protein to known toxins and allergens</td>
</tr>
<tr>
<td>• Glycosylated</td>
<td>✓</td>
<td>• Not glycosylated</td>
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</table>
Conclusions: TC1507 Maize

- Substantially equivalent to non-GM counterparts
- No evidence of food and feed safety concerns