“Tacogate”
Starlink Corn: A Risk Analysis

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Overview

- Biopesticides
  - History, regulations, modern applications
- Biotechnology and biopesticides
  - Plant-incorporated protectants (PIP’s) for crop protection
    - *Bacillus thuringiensis (Bt)*
      - From biopesticide to biotechnology (plant incorporated protectant)
- Risk assessment from dietary exposure
  - Starlink corn as example
What is a Pesticide?  What is a Biopesticide?

- **Pesticide:** a chemical intended to kill, injure, or repel a pest
  - Defined under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act)
    - Includes fungicides, insecticides, herbicides, rodenticides, antimicrobials, repellents

- **Biopesticide:** pesticides derived from natural materials
  - Regulated under FIFRA, FQPA (Food Quality Protection Act, 1996)
    - Plants
    - Microorganisms
    - Minerals
    - Animals
Classifications of Biopesticides

- **Microbial biopesticides**
  - *Bacillus thuringiensis*
  - *Aspergillus flavus*, strain AF 36

- **Plant-incorporated protectants (PIP’s)**
  - Applications of biotechnology
    - Genetic incorporation of chemical resistance factors or microbial pest control factors into plant tissues

- **Biochemical biopesticides**
  - Naturally occurring chemicals
  - Mechanisms other than direct toxicity
    - Sex pheromones (interfere with mating)
    - Repellent chemicals
Microbial Biopesticides: *Bacillus thuringiensis* as Example

- Most widely utilized and studied microbial pesticide
- Gram-positive, motile, rod-shaped bacterium
  - Ubiquitous (naturally occurring)
  - In environment, exists as a dormant (spore) form
    - Forms protein crystals (Cry) in spore form
  - Registered for use by U.S. EPA as biopesticide in U.S. since 1961
    - Crop protection, forestry, greenhouses, vector control, outdoor residential uses
**Bacillus thuringiensis (Bt) and Crystal Proteins (Insecticides)**

- Cry proteins (produced by Bt)
  - a.k.a. *delta*-endotoxin
- Insecticidal properties are selective for the insect
  - Contrast with other insecticides
  - After ingestion, Cry proteins are cleaved to form active insecticidal protein
    - Binds to receptors in insect midgut
    - Leads to cessation of insect feeding
  - Reasons for selectivity
    - Receptor selectivity (insects)
    - Alkaline environment required for cleavage to active protein
**Bt and Human Health Risks**

- **The toxicology pathway**
  - **Hazard** is ubiquitous
  - **Exposure** (contact) is not unusual
  - **Doses** are low (below threshold for response)
    - Results of mammalian, human studies
      - No effects at doses > 5,000 mg/kg Cry proteins
    - Contrast with other insecticides
  - **Response** follows the dose

- **Other (non-toxicological) risks from environmental, dietary exposure to Bt?**
Applications of biotechnology

Several important *Bt* genes encoding Cry proteins have been characterized

- Amino acid sequences, protein structure
- Modification of gene sequences can yield Cry proteins with enhanced insecticidal selectivity

Relevance of limiting target range
- Can Cry genes be introduced into plants to confer protection against specific target pests?
From *Bt* to Plant-Incorporated Protectant: Biotechnology

- **Methods of incorporation of Cry genes into plant genomes**
  - Microprojectile bombardment
    - Gene of interest is fixed to tungsten or gold particles (microcarriers)
    - Gene directly delivered to host cells at high speed (penetrating nucleus)
    - Limited efficiency
  - Agrobacterium-mediated transformation
    - *A. tumefaciens* (naturally occurring)
      - Plant pathogen
    - Plasmid gene of *A. tumefaciens* can incorporate into host genome, altering gene expression of the host
    - Cry genes can be introduced to this plasmid, and incorporated into host genome
Examples of Plant-Incorporated Protectants (Cry Proteins)

- Maize, potato, cotton
- Applications to corn
  - European corn borer as major pathogen of corn
- Modified Cry genes have been introduced into certain strains of corn (Bt corn)
  - Cry gene modifications are selective for the European corn borer
  - Incorporated gene remains stable in subsequent generations
  - Gene expression of Cry proteins (in target plant) is consistent when grown in different geographies
    - Highest expression in leaf of plant
**Bt Corn in the United States**

- Increasing utilization of Bt corn in U.S. agriculture since 1996
  - 1% in 1996 (.4 million acres)
  - 6% in 1997
  - 18% in 1998
  - 26% in 1999 (19.5 million acres)

- First Cry protein (as PIP) approved for use in corn (for human consumption) in 1995
**Bt corn: Risks and Benefits**

- Effective pest control
- Reduced use of conventional pesticides
- Reduced risk of mycotoxin contamination
  - Studies in Europe, United States
- Improved crop yields
- Pest resistance to Cry protein
- Effects on non-target organisms
- Transfer of gene to other plants
- Risk perception
  - Biotechnology and “Frankenfoods”
- Dietary risks from Cry proteins?
The Saga of Starlink Corn

- Genetically modified corn (PIP)
  - Carrying Cry9c gene and protein
  - Gene inserted to protect against European corn borer, cornstalk borer, corn earworm

- Manufacturer applied to U.S. EPA for use in animal feed and foods
  - 1998: EPA granted limited registration for animal feed (not for human consumption)
    - EPA planned for additional risk assessment
    - Rationale: distinctions of Cry9C from other insecticidal Cry proteins
      - Chemical stability (heat and acid)
      - Structural characteristics (allergenicity?)
The Saga of Starlink Corn

- 9/2000: Environmental group reported detection of Cry9c DNA (not protein product) in commercial foods
  - Taco shells
- Received significant media coverage
- Led to food recalls, and reassessment of risks by regulatory agencies
  - > 2.5 million boxes of taco shells recalled
    - High cost to manufacturer (millions$)
- Followed by reports of adverse food reactions associated with taco shells, other corn products to FDA
  - Reports suggestive of possible food allergy reactions
The Saga of Starlink Corn

What was unique about Starlink corn?

- Starlink was not first instance of human exposure to genetically modified plants
  - Another PIP (Cry1ab) had received regulatory approval for human consumption uses prior to Starlink (1995)
    - US EPA approved its use as PIP
    - No reports of adverse food reactions had been reported in association with dietary exposure
- Starlink was an example of PIP genes being discovered in food where it was not expected
  - And had not received regulatory approval
Adverse Reactions to Foods Derived from Starlink Corn?

- Reports of adverse food reactions followed quickly after media reports of Cry9c gene in taco shells
- CDC conducted a study of suspected cases
  - Case definition: suspected anaphylactic (allergic) reaction within 1 hour of consumption of corn-derived products
    - Hives, rash, swelling of mouth or throat
    - Vomiting, diarrhea, cramping involving only one individual
    - Symptoms not obviously explained by a pre-existing medical condition
  - Of 51 adverse reports, 28 met case definition
    - 24 participated in subsequent investigation
Investigation consisted of assessment of plausibility of allergic reaction to Cry9c protein
- Protein as hazard (in contrast to the gene encoding the protein)
- Distinctions between allergic responses and toxic responses
  - Hazard → Exposure → Dose → Response
  - Production of antibodies (immunoglobulin E- aka IgE) mediate the allergic response
    - In an allergic individual, the allergic response can occur from very low doses
Allergic Reactions to Foods Derived from Starlink Corn?

- Individuals with suspected allergic food reactions were tested for a specific IgE for the Cry9c protein
  - Positive antibody test would suggest Cry9c allergy as possible cause of adverse reaction
- Two other groups of individuals were tested for same antibody
  - Pooled blood samples from prior to 1996 (when Starlink was introduced)
    - Control group
    - Individuals with known history of food allergies
      - Tend to have higher production of many types of IgE
        - If positive for Cry9c IgE, would suggest plausibility of allergic reaction
Allergic Reactions to Foods Derived from Starlink Corn?

- Cry9c IgE antibodies were not detectable in any of the blood samples.
- Other IgE antibodies were detectable, but they were to common environmental allergens: Animal dander, peanuts, etc.
- Results did not confirm an allergic reaction to Cry9c protein as cause of reported adverse food reactions.

Starlink Corn: The Aftermath

- November, 2000
- EPA re-assessed potential allergenicity of Starlink corn
  - Accepted findings of CDC, but could not rule out the possibility of allergic reactions to Cry9c
    - Manufacturer of Starlink subsequently withdrew its petition for food registration
- EPA subsequently developed additional criteria for risk assessment of possible allergic responses to PIP’s
  - Resistance to acid treatment and protein digestion
  - Molecular weight range
  - Immunologic (IgE) responses in rat models
  - Detection of the protein product in the bloodstream (animal studies)
EPA responsible for registration of PIP’s
- USDA (Animal and Plant Health Inspection Service) oversees research and field trials of PIP’s
- US FDA provides input for PIP’s that may pose a risk to food, animal feed

All PIP’s that have been approved by US EPA were given time-limited registration
- Need for re-assessment of risks based upon additional scientific data

http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/1-overview.pdf
Plant-Incorporated Protectants: EPA Risk Assessment of *Bt* Corn

- 2 Bt corn PIP’s (Cry1Ab and Cry1F) re-assessed in 2001

- Results of risk re-assessment
  - Protein stability studies
    - Proteins inactivated by typical food processing procedures
    - Readily digestible, and degraded in gastric fluids
  - Acute oral toxicity data
    - No effects at acute oral doses > 4000 mg/kg
  - Met conditions for re-registration for food uses under Food Quality Protection Act
    - *Reasonable certainty of no harm* standard of safety
Ecological re-assessment of risk conducted

Risks of gene transfer? Risks to non-target species?
- Available (and new) data found no significant risk of gene capture, gene expression by wild or other corn products
  - Results were confirmed in studies by USDA
- No adverse effects observed in non-target species fed Bt corn as part of diet
  - Avian, honey bees, wasps, beetles, earthworms, monarch butterflies
- No effects observed on microbial populations in soil where PIP’s are grown
Plant-Incorporated Protectants: EPA Risk Assessment of Bt Corn

- Re-assessment of insect resistance was conducted
- Has Bt corn resulted in insect resistance to PIP’s?
  - Available and new data found no reported incidents of insect resistance to the Cry proteins in Bt corn
- EPA has mandated enhanced insect resistance management (IRM) programs as part of the registration process for Bt corn
- Both Bt corn varieties (Cry1Ab and Cry1F) were determined eligible for re-registration by EPA
**Bt corn: Risks and Benefits**

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Bt Corn: Conclusions

- PIP’s (and Starlink) as example of emerging challenges in biotechnology
  - New paradigms in health risk assessment
    - Toxicity vs. other health endpoints
    - Available data suggest that risks from Bt corn do not differ substantially from conventional corn varieties
      - Notion of substantial equivalence
  - Public perceptions relating to the biotechnology used to create food products
    - In contrast to the food itself
  - Risk assessment methods for food biotechnology will continue to evolve in the future
Questions for Discussion

- Do you consider *Bt* corn (PIP) to have different health risks than corn that has been treated with *Bacillus thuringiensis* as a biopesticide? Why?

- Has Starlink corn changed your perspective on the risks and benefits of genetically modified foods? How?