“Tacogate” Starlink Corn: A Risk Analysis

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Overview

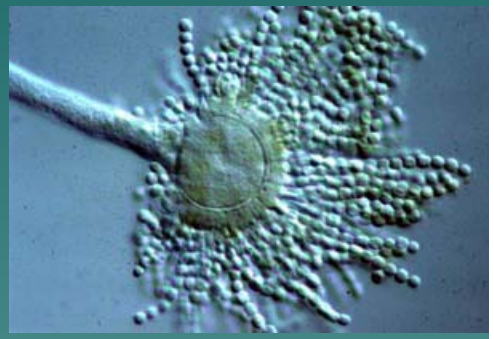
- Biopesticides
  - History, regulations, modern applications
- Biotechnology and biopesticides
  - Plant-incorporated protectants (PIP's) for crop protection
    - *Bacillus thuringiensis* (*Bt*)
      - From biopesticide to biotechnology (plant incorporated protectant)
- Risk assessment from dietary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

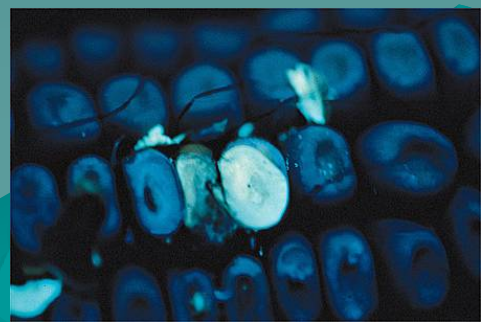
What is a Pesticide? What is a Biopesticide?

- 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

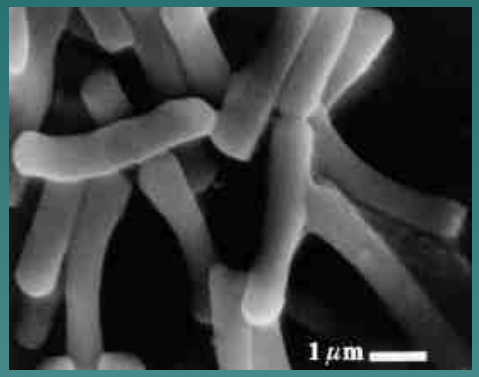
- Microbial biopesticides
  - *Bacillus thuringiensis*
  - *Aspergillus flavus*, strain AF 36
- Plant-incorporated protectants (PIP's)
  - Applications of biotechnology
    - Genetic incorporation of chemical resistance factors or microbial pest control factors into plant tissues
- Biochemical biopesticides
  - Naturally occurring chemicals
  - Mechanisms other than direct toxicity
    - Sex pheromones (interfere with mating)
    - Repellant chemicals

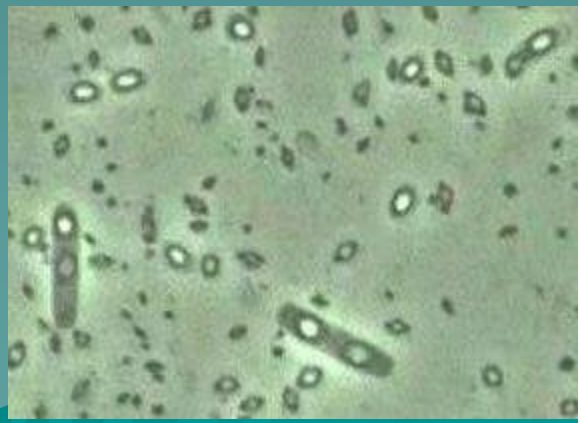




Microbial Biopesticides: *Bacillus thuringiensis* as Example

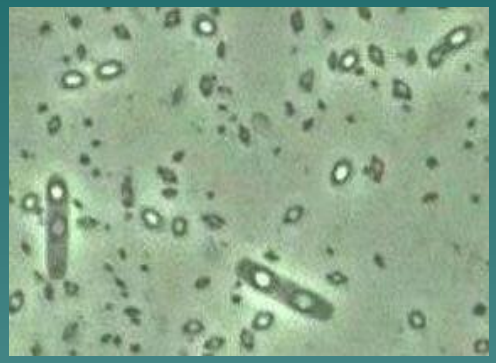
- Most widely utilized and studied microbial pesticide
- Gram-positive, motile, rod-shaped bacterium
  - Ubiquitous (naturally occurring)
  - In environment, exists as a dormant (spore) form
    - Forms protein crystals (Cry) in spore form
  - Registered for use by U.S. EPA as biopesticide in U.S. since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



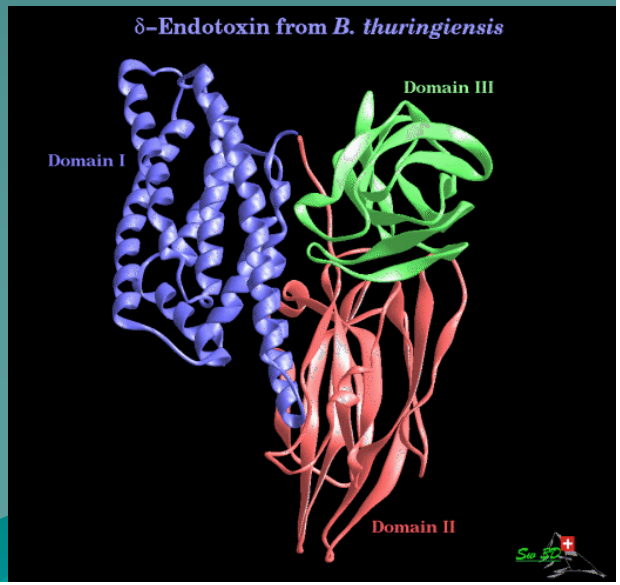


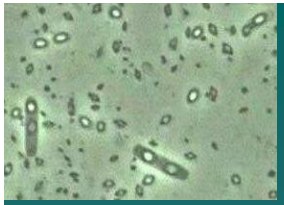
Bacillus thuringiensis (Bt) and Crystal Proteins (Insecticides)

- 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

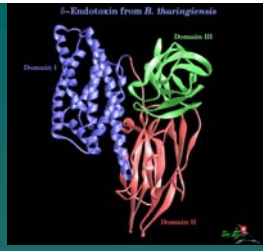


Cry Protein

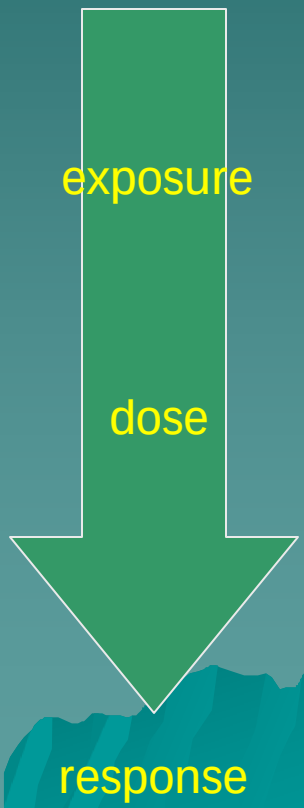




Bt and Human Health Risks



- 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?



From *Bt* to Plant-Incorporated Protectant

- 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?

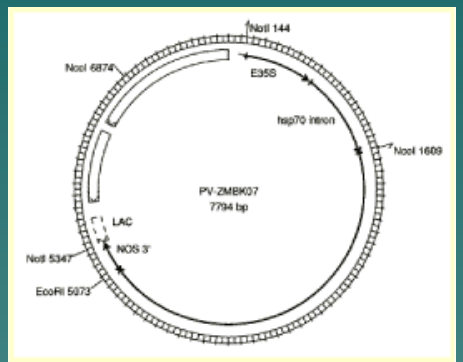
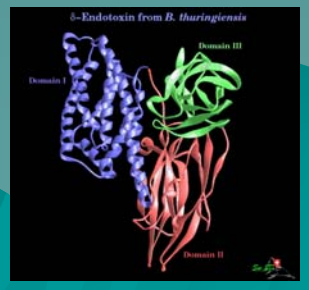
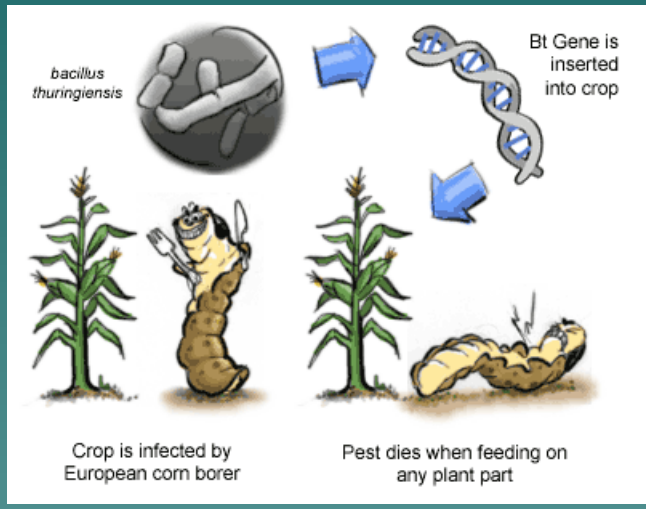


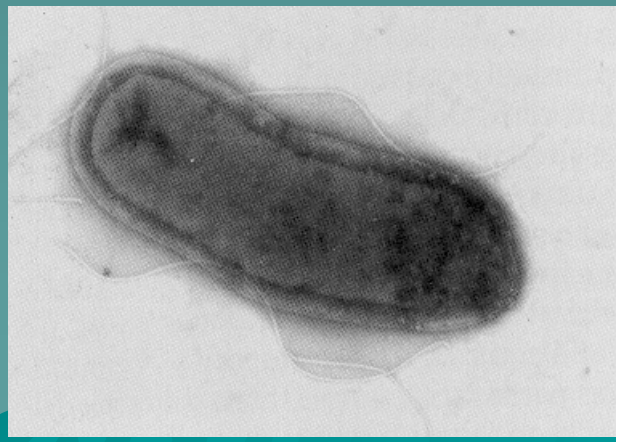
Fig. 1. Plasmid map of PV-ZMBK07. Restriction sites and their locations in base pairs, used during Southern analysis are shown.



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

Table 2. Summary of specific protein levels in MON 810 tissues harvested from 1995 European field trials.[1]

		Protein (µg/g fresh weight tissue)		
		Cry1Ab	CP4 EPSPS[2]	GOX[2]
Leaf	mean[3]	8.60	N.D.[4]	N.D.
	range[5]	7.59-9.39	N.A.[6]	N.A.
Forage[7]	mean	6.08	N.D.	N.D.
	range	4.21-9.23	N.A.	N.A.
Grain[8]	mean	0.53	N.D.	N.D.
	range	0.42-0.69	N.A.	N.A.

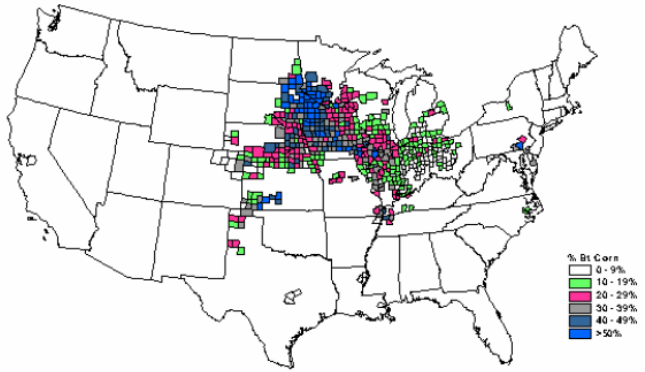
1. All values are expressed as µg protein / g fresh weight tissue.
2. Molecular analysis demonstrated that CP4 EPSPS and *gox* genes were not present in MON 810.
3. The means were calculated from the analysis of a single pooled sample from 5 sites.
4. Not detected.
5. The range is the minimum and maximum values from the analysis of samples from 5 sites.
6. Not applicable. Protein was not detected, therefore no range.
7. The mean and range were calculated from the analysis of two pooled plants from 4 sites.
8. The mean and range were calculated from the analysis of pooled ears from 4 sites.

Table 1. Summary of specific protein levels in MON 810 tissues harvested from 1994 US field trials.[1,2]

					Overseason Leaf		
Protein	Leaf	Grain	Whole Plant[3,4]	Pollen[3]	1st	2nd	3rd
Cry1Ab	9.35	0.31	4.15	0.09	9.78	8.43	4.91
CP4 EPSPS	N.D.[5]	N.D.	N.D.	N.A.[6]			
GOX	N.D.	N.D.	N.D.	N.A.			
NPTII	N.A.	N.A.	N.A.	N.A.			

1. The means were calculated from the analysis of six plant samples, one from each of six trial sites, unless otherwise indicated.
2. All values are expressed as µg protein / g fresh weight tissue.
3. The mean values were calculated from analyses of plant samples from one site.
4. The mean values were calculated from analyses of two replicate samples from one site.
5. Not detected.
6. Not analyzed.

Figure 1. U.S. Distribution of *Bt* Corn*

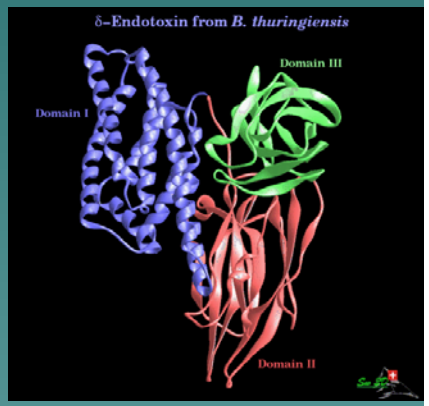


Bt corn: Risks and Benefits

- 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

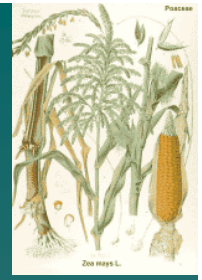
- Genetically modified corn (PIP)
  - Carrying Cry9c gene and protein
  - Gene inserted to protect against European corn borer, cornstalk borer, corn earworm
- Manufacturer applied to U.S. EPA for use in animal feed and foods
  - 1998: EPA granted limited registration for animal feed (not for human consumption)
    - 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



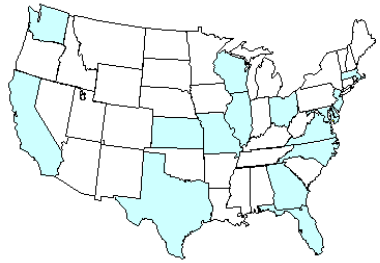
- What was unique about about Starlink corn?
  - Starlink was not first instance of human exposure to genetically modified plants
    - Another PIP (Cry1ab) had received regulatory approval for human consumption uses prior to Starlink (1995)
      - US EPA approved its use as PIP
      - No reports of 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

CDC

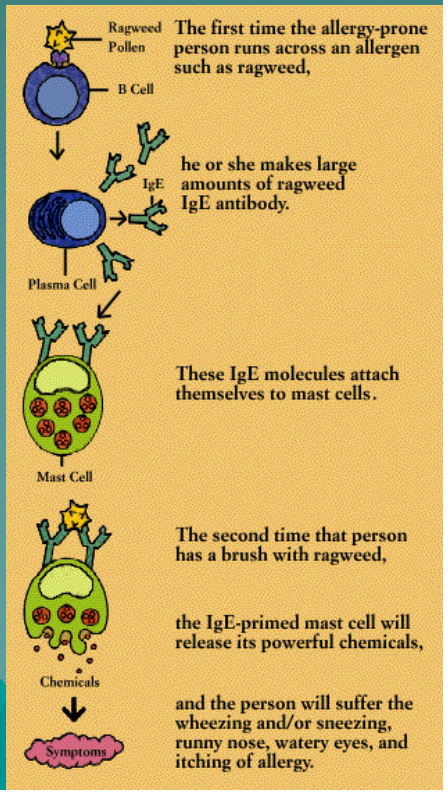
Figure 1. Distribution of Adverse Event Reports Made to FDA That Met CDC Case Definition for Possible Allergic Reaction Following a Meal Containing a Corn Product



Allergic Reactions to Foods Derived from Starlink Corn?

?

- 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

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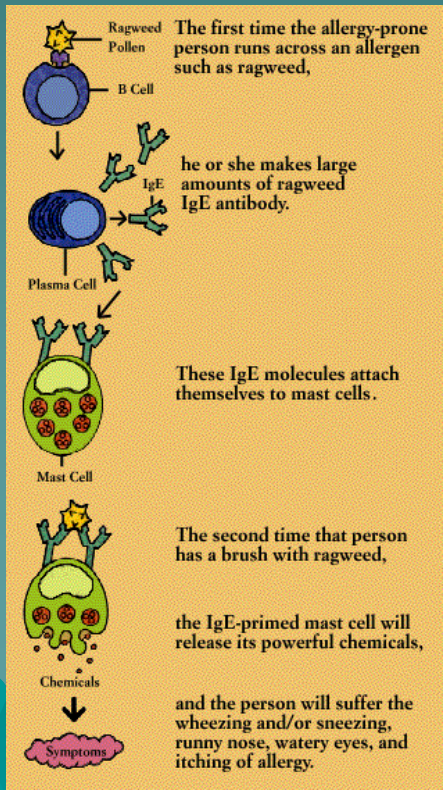
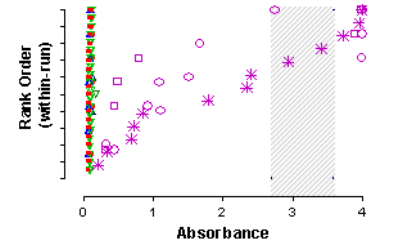


http://www.cdc.gov/nceh/ehhe/Cry9cReport/default.htm

Allergic Reactions to Foods Derived from Starlink Corn?

- Investigation results
  - 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

Figure 5. Overall Depiction of FDA ELISA Results: IgE Reactivity to Cry9c in Cases, Pre-1996 Controls, and Atopic Controls and IgE Reactivity to Cat, Grass, and Peanut Allergens in Positive Controls



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)

Bt Plant-Incorporated Protectants, 2004

- 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

Plant-Incorporated Protectants: EPA Risk Assessment of *Bt* Corn

- 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

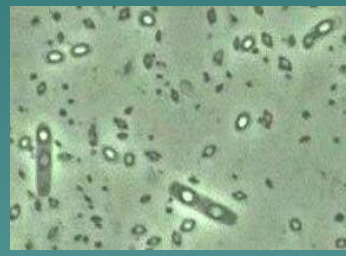
- Re-assessment of insect resistance was conducted
- Has Bt corn resulted in insect resistance to PIP's?
  - Available and new data found no reported incidents of insect resistance to the Cry proteins in Bt corn
- EPA has mandated enhanced insect resistance management (IRM) programs as part of the registration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

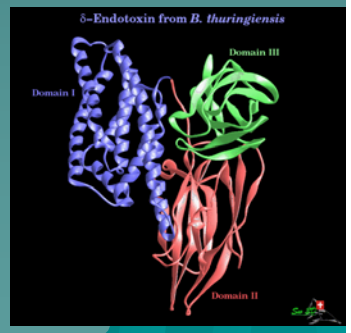
Bt corn: Risks and Benefits

- 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?

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?