US EPA Biopesticide Regulation

- U.S. EPA Office of Pesticide Programs
  - Registration Division & Science Divisions
    - Conventional chemical pesticides
  - Biopesticides & Pollution Prevention Division
    - multidisciplinary: Review Managers and Scientists
    - Biochemical Pesticides
    - Microbial Pesticides
    - Plant-Incorporated Protectants
  - www.epa.gov/pesticides/biopesticides
Biopesticides & Pollution Prevention Division

• Division Director: Janet Andersen
• Microbial Pesticide Branch Chief: Dennis Szuhay

• Senior Scientists
  – Toxicology/human health: John Kough
  – Ecological effects: Zigfridas Vaituzis

• Specialist scientists
  – Gene flow: Tessa Milofsky
  – Insect Resistance Management: Sharlene Matten
Plant Incorporated Protectants

- Regulated by USDA, FDA, and EPA
- New 40 CFR part 174
- System based on Sound Science and Transparency
- Regulation of both the Gene and the Pesticide Produced
- All Proteins thus far
Pesticides

- US Pesticide = any substance intended for mitigating a pest or intended for use as a plant regulator, defoliant, or desiccant
- Plant Incorporated Protectants = a pesticidal substance that is intended to be produced and used in a living plant, and the genetic material to produce it.
PIPs Process

- Transparency
- All applications announced in FR
- All non-cbi data publicly available during the review process
- Numerous public meetings and Science Advisory Panels
- Extensive Use of Website
Risk Assessment

- Hazard
  - Toxicity

- Exposure
  - Scale of use, use patterns, application rates
  - Persistence, degradation, mobility
  - Population dynamics, infectivity, gene transfer

- Risk
  - Non-targets, humans, endangered species, domestic animals
Data Submission for Protein Plant-Pesticides

- **Product Characterization**
  - source of the gene and expression system
  - nature of pesticidal substance
  - modifications to introduced trait
  - biology of recipient plant, stability of the introduced traits
  - test substance equivalence

- **Toxicology**
  - acute oral toxicity
  - mode of action
  - allergenicity screening

- **Exposure & Non-target Effects**
Testing of PIPs

- Product characterization
  - Source of the genes
  - What protein(s) are made
  - Expression - In what parts of the plant are the proteins found and at what level
  - Includes review of any marker genes
Testing PIPs (continued)

• Human health
  – Exact structure of the protein including amino acid sequence
  – Similarity of this protein to any known toxin or allergen
  – Toxicity testing—feeding the protein to mice
  – Stability/breakdown of protein in digestive fluids
Testing PIPs (continued)

- Ecological effects
  - Effects on non-target organisms
    - birds
    - fish
    - aquatics
    - invertebrates
  - Endangered Species
  - Monitoring
Testing PIPs (continued)

- Environmental fate
  - Protein expression
  - Protein persistence and degradation
  - Gene transfer
EPA Tox-Assessment Data
Mode of Action: Pore Forming Toxin

Cry1Ab

Swiss-3Dimage
EPA Eco-Assessment Data

• Cry1Ab
  – Acute oral mice
    • no effect >4000mg/kg
    • no effect >3280mg/kg
    • no effect >5050mg/kg
  – Acute oral quail
    • no effect >100,000ppm (Corn meal)
    • no effect >2000mg
  – Catfish
    • no effect at 100% of diet (Corn meal)
EPA Eco-Assessment Data

- Cry1Ab (continued)
  - Daphnia
    - no effect > 150mg/l
  - Green lacewing larvae
    - no effect > 20ppm
  - Parasitic wasp
    - no effect > 20ppm
  - Ladybird beetle
    - no effect > 20ppm
    - no effect (in pollen)
EPA Eco-Assessment Data

• Cry1Ab (continued)
  – Honey bee
    • Adult no effect > 20ppm
    • Larvae no effect > 20ppm
    • Larvae no effect (in pollen)
  – Collembola
    • no effect > 200ppm
    • LD$_{50}$ 240mg/kg/soil  NOEL 125mg/kg/soil
  – Earthworm
    • nontoxic > 200ppm
    • nontoxic
EPA Eco-Assessment Data

• Cry1Ab Expression Data
  – Degradation – Bioactivity half life
    • Pure Cry1Ab plus soil 8.3 days
    • Cry1Ab expressed in corn - 25.6 days
    • Cry1Ab in corn plus soil 1.6 days
EPA Eco-Assessment Data

• Cry1Ab Expression Data
  – Expression levels – avg. protein ug/g fresh tissue from corn

<table>
<thead>
<tr>
<th></th>
<th>Lab</th>
<th>Field</th>
<th>Construct 1 - Highest levels in seedlings, declined thereafter through senescence. No detectable levels in silage.</th>
<th>Construct 2 – Highest levels in leaves at physiological maturity (25 days), declining thereafter.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>1.3</td>
<td>9.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>Not detectable</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>0.57</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>Grain</td>
<td>-</td>
<td>0.31</td>
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<tr>
<td>Whole plant</td>
<td>1.77</td>
<td>4.15</td>
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</tbody>
</table>
Testing PIPs (continued)

- Resistance Management
  - EPA requires an insect resistance management (IRM) plan for each Bt product
  - Required reports on compliance and adoption
  - Research and monitoring requirements
Refugia and Buffers

- Side by side refuge

- Separated refuge

- Refuge combined with barriers
Cotton/B.t.

- **Hazard assessment**
  - Non-target species
    - Nontoxic to birds
    - Nontoxic to beneficial insects
      - wasps, lacewings, honeybees, ladybird beetles

- **Exposure Assessment**
  - no exposure to endangered species
  - estimated 1.44gm protein in soil/acre
  - undetectable in cottonseed meal
  - almost undetectable in pollen
  - not detectable in nectar
  - gene transfer to other plants (cotton)
    - wild/feral cotton: Arizona-sterile offspring
    - Hawaii, Everglades & Florida Keys
      - labeling: no commercial use
## Registered Products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Plant Type</th>
<th>Company</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt Cry3A</td>
<td>Potato</td>
<td>Monsanto</td>
<td>1995</td>
</tr>
<tr>
<td>Bt Cry1Ac</td>
<td>Cotton</td>
<td>Monsanto</td>
<td>1995</td>
</tr>
<tr>
<td>Bt Cry1Ab</td>
<td>Corn</td>
<td>Syngenta</td>
<td>1996/8</td>
</tr>
<tr>
<td>Bt Cry1Ab</td>
<td>Corn</td>
<td>Monsanto</td>
<td>1996</td>
</tr>
<tr>
<td>PLRV replicase/Cry3A</td>
<td>Potato</td>
<td>Monsanto</td>
<td>1998</td>
</tr>
<tr>
<td>Bt Cry1F</td>
<td>Corn</td>
<td>Dow/Mycogen</td>
<td>2001</td>
</tr>
<tr>
<td>Bt Cry1F</td>
<td>Corn</td>
<td>Pioneer/Dupont</td>
<td>2001</td>
</tr>
<tr>
<td>Bt Cry2Ab + Cry1Ac</td>
<td>Cotton</td>
<td>Monsanto</td>
<td>2002</td>
</tr>
<tr>
<td>Bt Cry3Bb1</td>
<td>Corn</td>
<td>Monsanto</td>
<td>2003</td>
</tr>
<tr>
<td>Bt Cry3Bb1 + Cry1Ab</td>
<td>Corn</td>
<td>Monsanto</td>
<td>2003</td>
</tr>
<tr>
<td>Bt Cry1Ac + Cry1F</td>
<td>Cotton</td>
<td>Dow</td>
<td>2004</td>
</tr>
<tr>
<td>Bt moCry1F</td>
<td>Corn</td>
<td>Dow/Mycogen</td>
<td>2005</td>
</tr>
<tr>
<td>Bt Cry34Ab1 + Cry35Ab1</td>
<td>Corn</td>
<td>Monsanto</td>
<td>2005</td>
</tr>
</tbody>
</table>
Benefits example

- Corn Rootworm control is the highest insecticide usage in the U.S.
- Many corn rootworm pesticides are of concern to the Agency. Incidents, Restricted Use Pesticides
- New Bt Corn Rootworm products offers substantial environmental benefits (Cry3Bb1 & Cry 34/35)
- Syngenta also has a pending corn rootworm product and there are two pending multiple pest products from Dow & Pioneer.
Conclusions

• EPA has a strong regulatory review process
  – Transparency
  – Sound Science

• EPA’s web site and other efforts keep the public informed about its decisions
  http://www.epa.gov/pesticides/

• The US Biotech Database lists products
  http://usbiotechtechreg Nbii.gov/