Biotechnology Protein Safety Assessment: A GM Case Study

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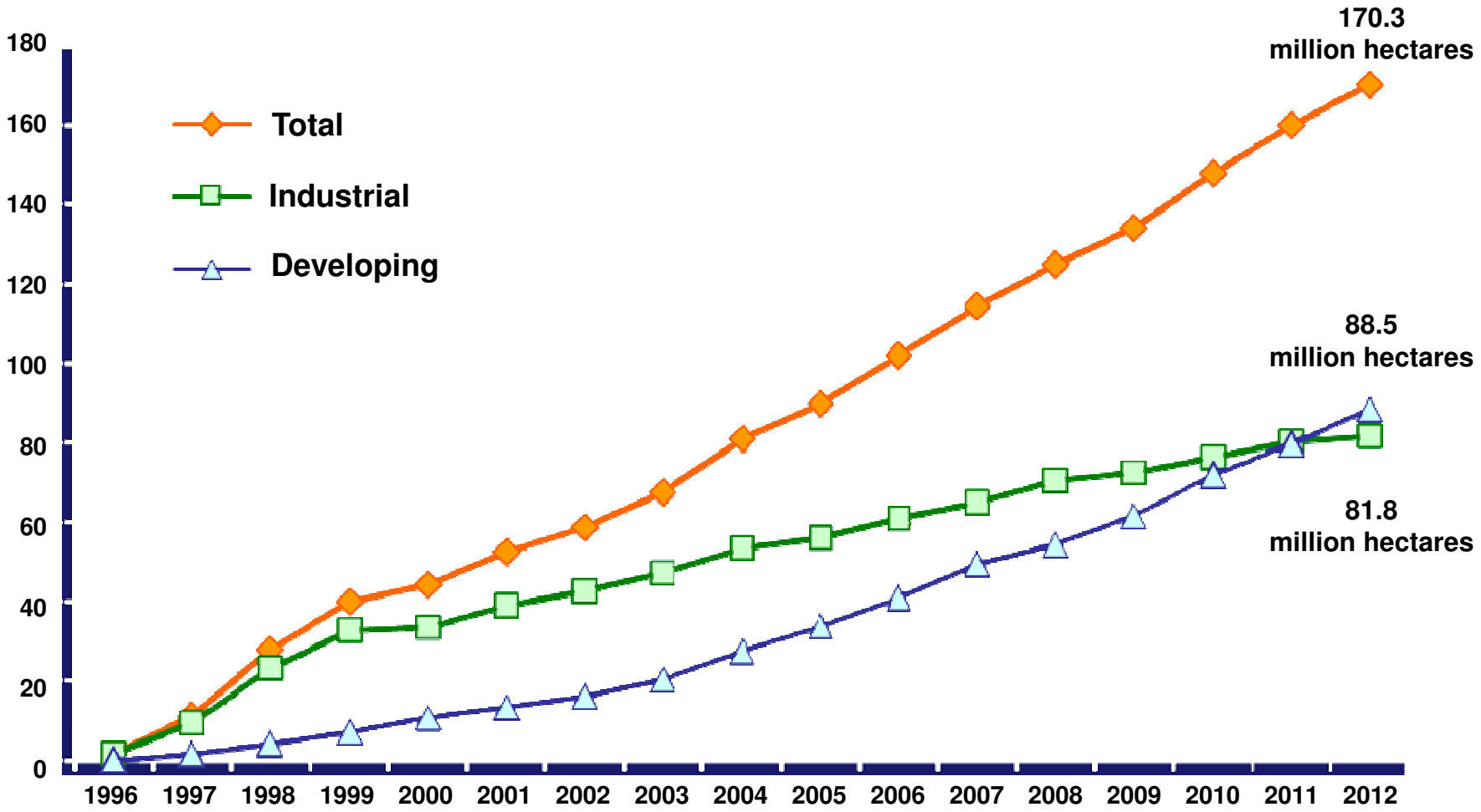
In 2012 the total area planted globally with biotech crops increased 6% to 170 million hectares



Source: James, C., 2012

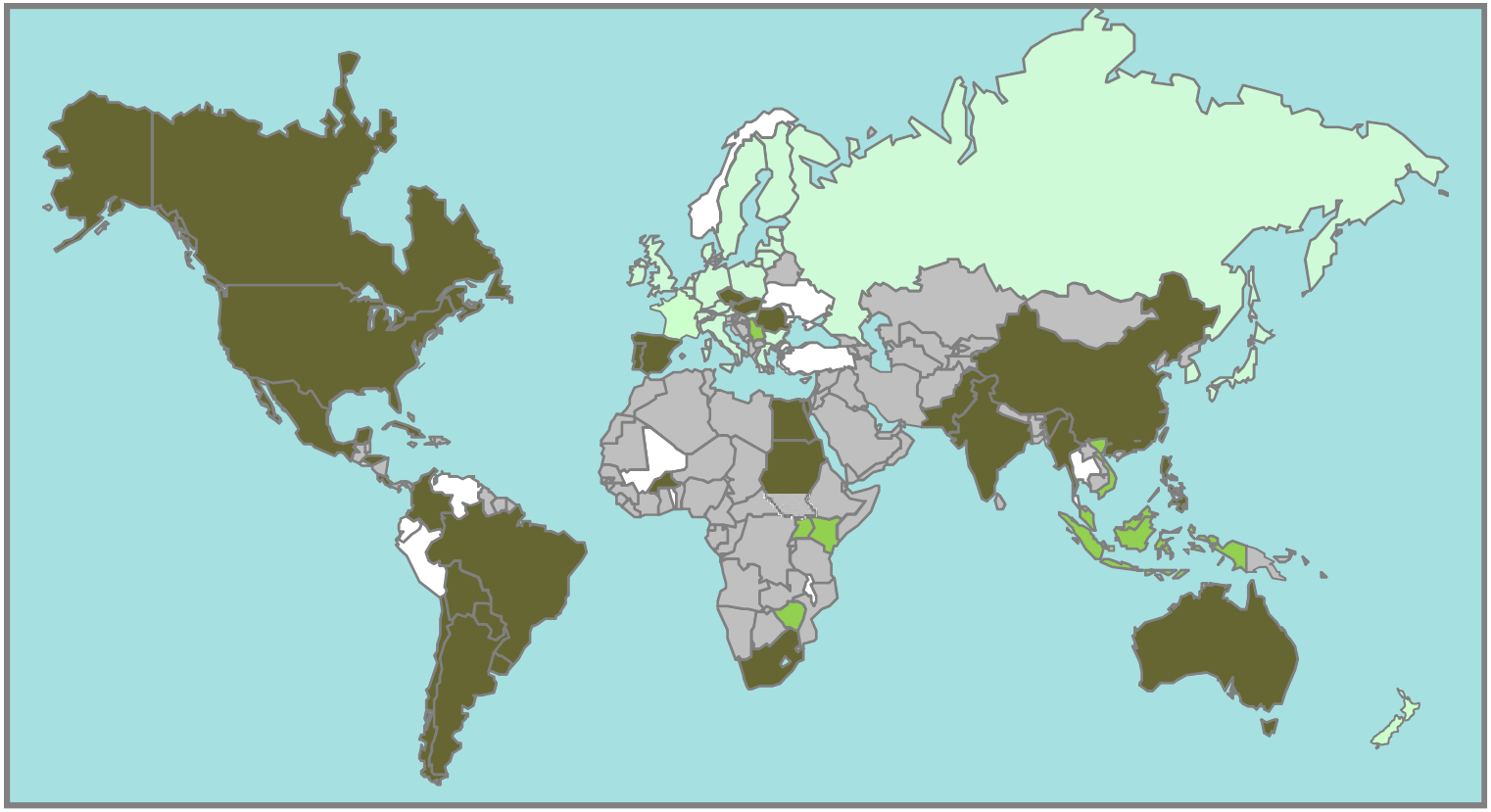
Adoption of GM crops has economic and environmental benefits

- Adoption of GM crops has resulted in:
  - Reduction in insecticide (213 million kg*) and herbicide (230 million kg*) active ingredient use
  - Increased use of more environmentally benign products
  - Decreased fuel consumption
  - Decreased green house gas emissions
  - Changing in farming systems

* Estimated total reduction from 1996-2010.

Source: Brookes, G and Barfoot, P., 2012

Regulatory Systems have been established throughout the world



Source: James, C., 2012 and Monsanto

Overview of the safety assessment of biotech crops

- The safety assessment includes:
  - Detailed characterization of the insertion
  - Characterization of the inserted protein
    - History of Safe Use
    - Allergy Safety Assessment
    - Protein Safety Assessment
  - Comparison of composition and nutritional components
  - Phenotypic and ecological assessments

Roundup Ready® 2 Yield soybeans

- Roundup Ready® 2 Yield soybeans are a second generation weed control product
  - Provide *in planta* tolerance to Roundup® brand agricultural herbicides
- Roundup Ready® 2 Yield soybeans provide herbicide tolerance by expressing the CP4 EPSPS protein
  - CP4 EPSPS is derived from *Agrobacterium* sp strain CP4

History of Safe Use: CP4 EPSPS

- Mode of Action is well understood
  - EPSPS enzymes catalyze a key step in the biosynthesis of aromatic amino acids
  - Roundup® brand herbicides bind to endogenous EPSPS proteins which inhibit amino acid production and results in cell death
  - CP4 EPSPS protein functions like other endogenous EPSPS enzymes, however it has a reduced affinity for Roundup® herbicide binding
    - The reduced affinity allows CP4 EPSPS to continue aromatic amino acid biosynthesis in the presence of Roundup® herbicides
    - The ability to continue amino acid biosynthesis confers tolerance

History of Safe Use: CP4 EPSPS

- CP4 EPSPS is structurally and functionally similar to other EPSPS proteins safely consumed in food and feed
  - EPSPS enzymes are ubiquitous in plants and microorganisms and are safely consumed
  - CP4 EPSPS is functionally equivalent to other food derived EPSPS enzymes except for its tolerance to Roundup® herbicides
- A number of CP4 EPSPS containing crops have been commercialized (first in 1996) and no adverse effects have been documented

Allergy Safety Assessment for Biotech Crops

- Purpose: Determine if the inserted protein is a known allergen, is cross reactive with a known allergen, or could become an allergen
  - No definitive test of protein allergenicity exists
  - 'Weight of evidence' approach recommended by Codex guidelines is used to determine the allergenic potential of the inserted protein
    - Is the protein from an allergenic source?
    - Is the protein similar to known allergens?
    - What is the exposure to the protein?

Summary of the Allergy Assessment

- Does the protein originate from an allergenic source?
  - CP4 EPSPS protein originates from *Agrobacterium*, which is not considered an allergenic source
- Does the CP4 EPSPS protein have sequence similarity to any known allergens?
  - No similarity with any allergens at the:
    - ≥35% identity over 80 or greater amino acids threshold
    - E score cut-off of ≤1e-5
    - No matching contiguous 8 amino acid segments
  - Allergen database (allergenonline.org) has been established and is updated annually and curated by international academic allergy experts
    - Bioinformatics assessment is repeated annually

Summary of the Allergy Assessment

- What is the potential exposure to the inserted protein?
  - CP4 EPSPS is expressed in very low levels in the grain (less than 0.05% of total protein)
  - CP4 EPSPS is readily digested in SGF
    - >98% of CP4-EPSPS was digested within 15 seconds

- **Conclusion: CP4 EPSPS does not pose a significant allergenic risk.**

Summary of Protein Safety Assessment

- Is the donor organism safe?
  - *Agrobacterium* species are not known for human or animal pathogenicity and are not known to be allergenic
- Does the inserted protein have a history of safe use?
  - CP4 EPSPS is structurally and functionally similar to other EPSPS proteins safely consumed in food and feed
  - CP4 EPSPS containing crops have been commercialized (first in 1996) and no adverse effects have been documented

Summary of Protein Safety Assessment

- Does the inserted protein exert toxicity?
  - Bioinformatics comparison demonstrates that CP4 EPSPS is highly unlikely to share any structural homology to any known toxins
  - As a confirmation CP4 EPSPS was administered as a single dose by gavage to mice at 572 mg/kg and had no adverse effects

- **Conclusion: No evidence that CP4 EPSPS is likely to pose a safety risk.**

Summary of the Characterization of CP4 EPSPS

- CP4 EPSPS has a well characterized mode of action
- CP4 EPSPS has a long history of safe use
  - CP4 EPSPS is structurally and functionally similar to other EPSPS proteins safely consumed in food and feed
  - CP4 EPSPS containing crops have been commercialized (first in 1996) and no adverse effects have been documented
- CP4 EPSPS has no sequence similarity to known allergens
- CP4 EPSPS is readily digested in SGF
- CP4 EPSPS does not exert toxicity
- **Conclusion: There is no evidence that CP4 EPSPS is likely to pose a safety or allergenic risk.**

References Cited in this Presentation

- Brookes, G and Barfoot, P. (2012). Global Impact of Biotech Crops, Environmental effects, 1996-2010. GM Crops and Food: Biotechnology in Agriculture and the Food Chain 3:2, 129-137.
- James, C. (2012). ISAAA Brief 44, Global Status of Commercialized Biotech/GM Crops: 2012.


THANK YOU