Agricultural Plant Biotechnology Impact on Animals

The Agricultural Biotechnology Stewardship Technical Committee

November, 2000

AGENDA

- The Promise of Plant Biotechnology
- U. S . Safety and Regulatory System
- Animal Feed Performance
- DNA and protein detection

The Promise of Plant Biotechnology

Agricultural Biotechnology

"Too many opponents of biotechnology willfully choose to emphasize the highly unlikely potential risks rather than recognize the years of experience, research and regulatory oversight that govern the safe use of these new technologies."

Norman Borlaug,
Nobel Peace Prize Laureate
Founder, World Food Prize

Biotechnology Overview

What is Biotechnology?

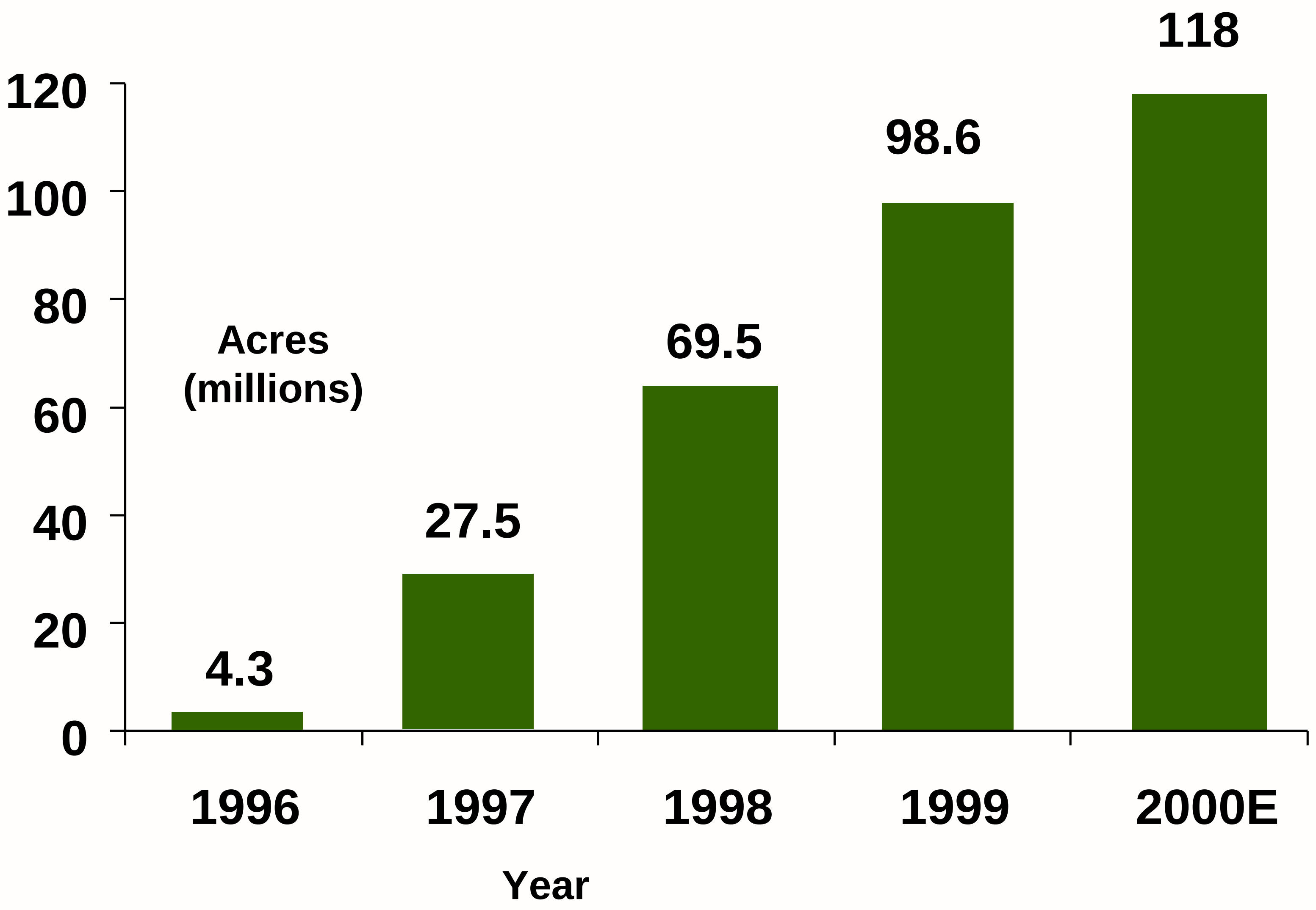
- The "application of living organisms to develop new products and processes" and " making use of the natural processes or products of living things." John Innes Centre, UK
- "A broad term that applies to all practical uses of living organisms -- anything from microorganisms used in the fermentation of beer to the most sophisticated application of gene therapy." The Union of Concerned Scientists, US
- "The use of living organisms or their products for commercial purposes." North Hardin (Kentucky) High School Technology Student Association

Agricultural Biotechnology
Why biotechnology?

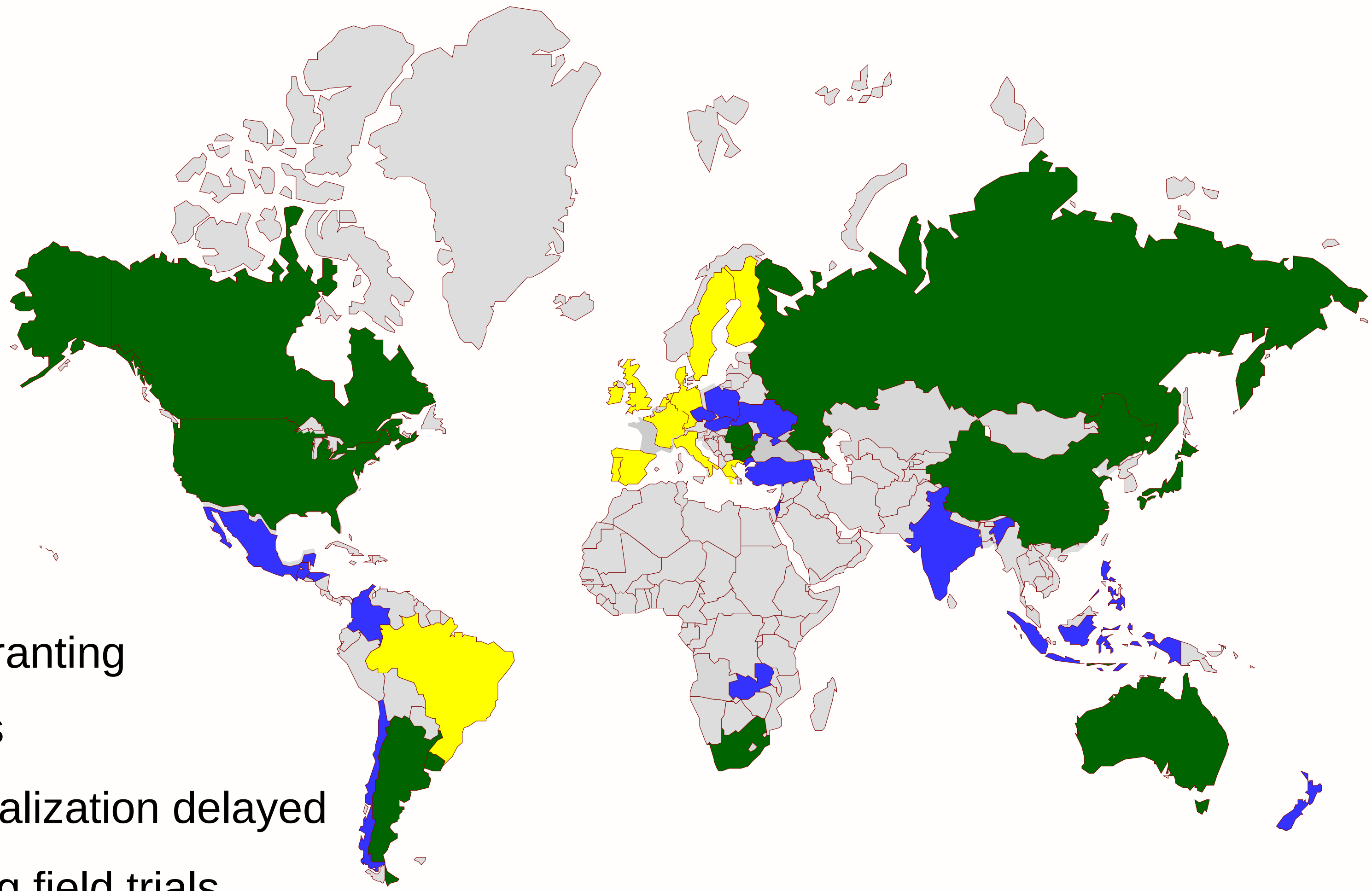
- World population is expected to increase to 10-12B by 2050 from 6B today, mostly in poorer regions.
- Farmable land is not expected to increase and probably will decrease.
- So how will farmers feed this huge population?
  - Higher yields
  - Reduced losses from pests
  - More nutritious food

Through Biotechnology

Worldwide Biotech Acres Continue to Expand



Global Regulatory Situation



- Actively granting approvals
- Commercialization delayed
- Conducting field trials

1999 key Commodity Crop Approvals in Select Markets:

	US	Canada	EU	Japan
Corn	16	11	4	13
Soy	11	4	1	2
Canola	6	18	3	12
Potato	17	17	0	2
Cotton	5	3	0	1
Beet	2	0	0	1

1999 Planting for Biotech Crops

- 27% of 1999 U.S. corn acreage planted with biotech seed products
- 54% of 1999 U.S. soybean acreage planted with biotech seed products
- 55% of 1999 U.S. cotton acreage planted with biotech seed products
- 70% of 1999 Canadian canola acreage planted with biotech seed products
- Multiple international markets planting biotech seed products

Sources: NCGA/ASA

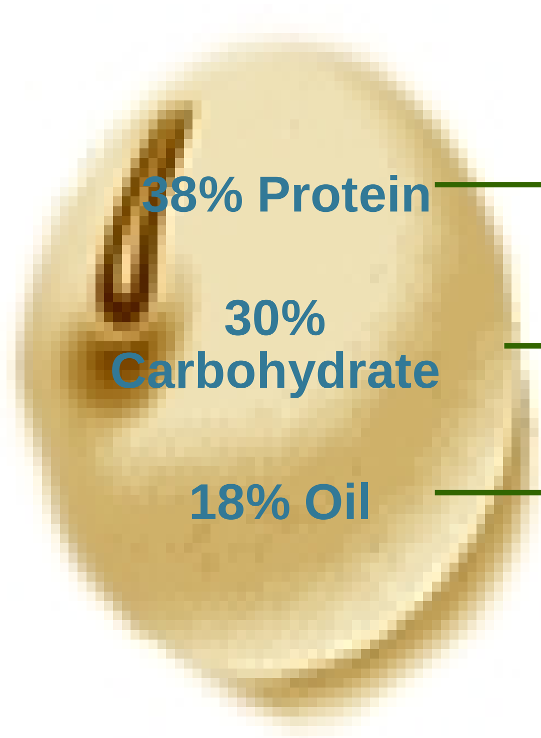
2000 Planting for Biotech Crops

- 25% of 2000 U.S. corn acreage planted with biotech seed products
- 54% of 2000 U.S. soybean acreage planted with biotech seed products
- 61% of 2000 U.S. cotton acreage planted with biotech seed products
- 70% of 2000 Canadian canola acreage planted with biotech seed products
- Multiple international markets planting biotech seed products:
  - 90% of soybeans in Argentina; 10-30% in Brazil*****
  - Adoption in China, former Soviet Union, India

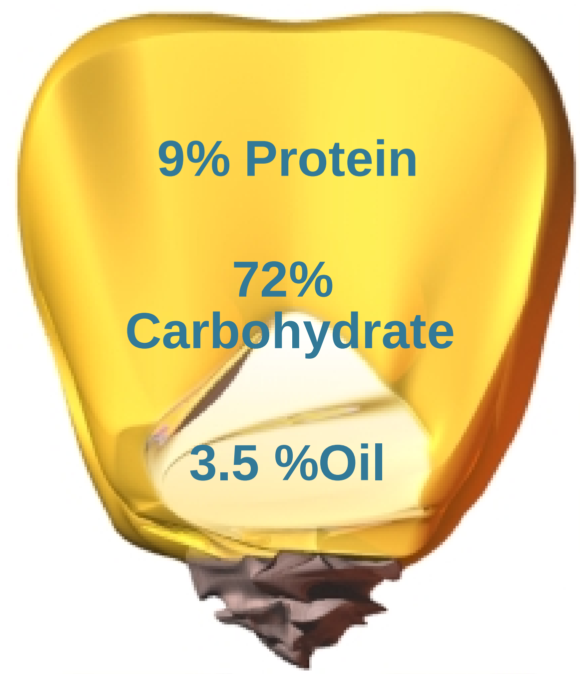
Sources: USDA/FAS

Improving Grain Quality Through Biotechnology

Soybean



Corn



Protein Quality
Protein Content

Energy, Digestibility
Modified Starches

Increased Stability
Healthy Oils
Industrial Feedstocks

Agricultural Biotechnology
Current Products:

- Reduced costs to producers:
  - Round-up Ready technology has lowered herbicide costs on average $12/acre
- Provided choices:
  - Plant-based Bt technology (insect protection) gives producers additional options to control pest (ECB) that costs industry up to $ 1 billion a year
  - Better food products:
  - Fruits and vegetables that are sweeter and firmer.
  - Healthier oils

A promising benefit of Bt corn for end users: decreased risk of reduced grain quality caused by fumonisin.

G.P. Munkvold and co-workers, Iowa State University

http://www.scisoc.org/feature/btcorn/TOP.html

Current Biotech Input Trait Products

Commercial Products	Benefits to Growers / Consumers
➢ Herbicide Tolerance (corn, soy, cotton, canola)	- Lower grower cost - Reduced herbicide residues - Enables no-till - Simplicity / flexibility
➢ Insect/Corn Borer Resistance (corn, cotton, potato)	- Lower grower costs - Reduced pesticide usage - Decreased fumonisin - Higher yields - Simplicity
➢ Virus Resistance (potato, papaya)	- Lower cost - Higher quality foods - Less acres used
➢ Delayed Ripening	- Higher quality food products - Longer shelf-life

Pipeline for Future Biotech Input Trait Products

Products / Traits	Benefits to Growers / Consumers
➢ Corn Rootworm Resistance	- Lower grower costs - Reduced pesticides - Yield
➢ Drought & Stress Tolerance	- Higher yields - Less risk - Less water & acres used - Production under hostile climate and soil conditions
➢ Higher Grain / Seed Yields	- Grain availability: decrease in acres needed

Pipeline for Future Biotech Input Trait Products

Products / Traits	Benefits to Growers / Consumers
• Corn Rootworm Resistance	- Lower grower costs - Reduced pesticides - Yield
• Less bound phosphorus	- Less phosphorus in animal waste entering the environment
• Drought & Stress Tolerance	- Higher yields - Less risk - Less water & acres used - Production under hostile climate and soil conditions

Agricultural Biotechnology
Near-future Products

- Nearly a limitless spectrum of foods, feeds, bio-fuels, bio-based materials and nutraceuticals.

  - Golden rice with beta-carotene and higher levels of iron
  - Corn which helps reduce harmful elements in animal waste
  - Stress resistant crops that grow in harsh conditions.
  - Nutritionally balanced grain
  - Pharmaceutical and vaccine production <u>and</u> delivery
  - Plants used as factories to produce chemical feedstocks and plastics

Pipeline for Future Biotech Output Trait Products

Products / Traits	**Benefits to Growers / Consumers**
➢ Foods richer in nutrients and vitamins	- Better health & nutrition - Night blindness cure
➢ Less bound phosphorus (corn, soy)	- Less phosphorus in animal waste entering the environment - Less cholesterol in meat
➢ More digestible starch/ energy	- Improved efficiency - Less waste - Less grain energy processing
➢ Corn BioPolymers/ plastics	- Lower cost renewable resource - New crop market
➢ Pharma intermediates/ proteins	- Lower cost pharmaceuticals - New crop market
➢ Anti-disease/fungal/ bacterial agents	- Reduced medical care - Less fungicide & antibiotic - Use reduced mycotoxin
contamination of	feeds/foods

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Pipeline for Future Biotech Output Trait Products

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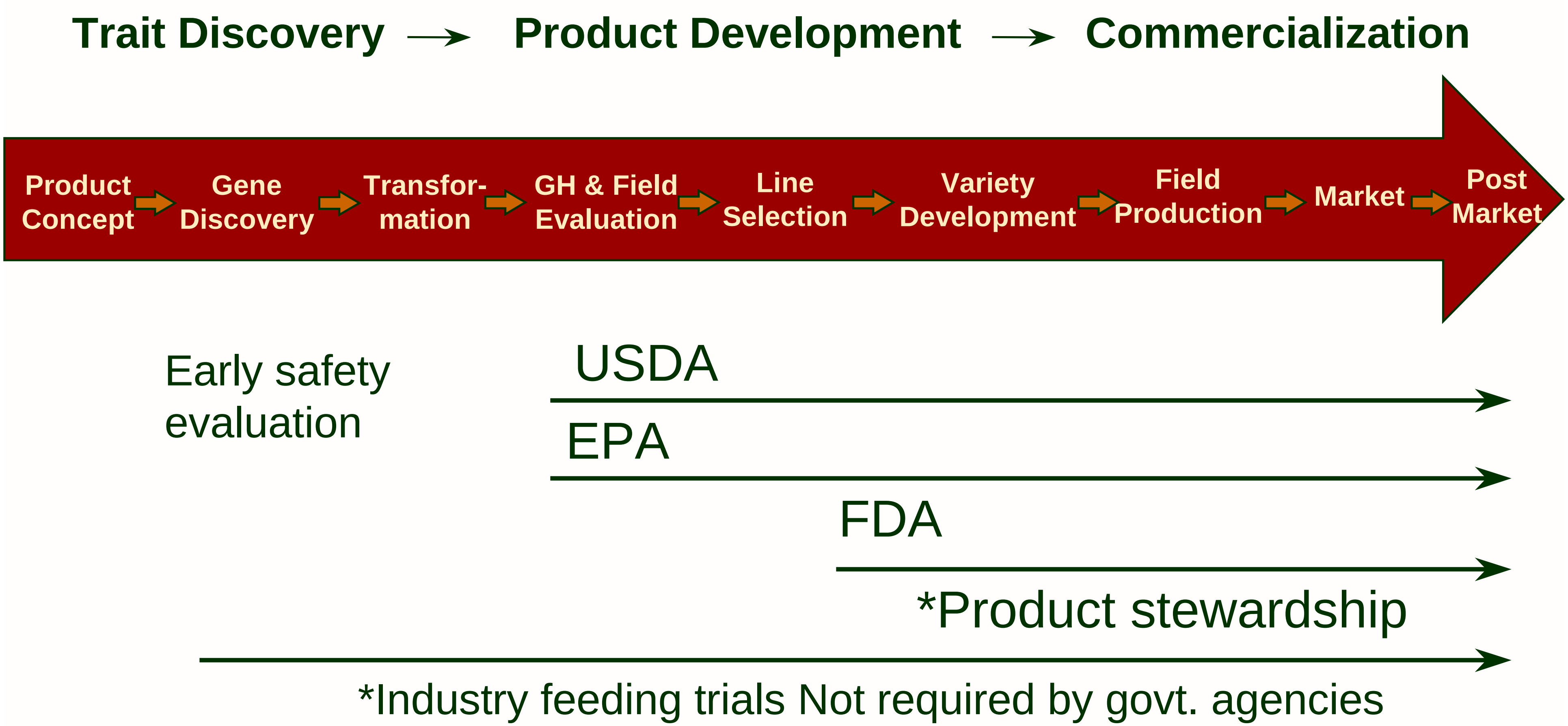
contamination of	feeds/foods

Questions from the feed and food industry

- Is biotech grain safe for feed?
- Is the feed performance the same between biotech crops and conventional crops?
- Are the proteins/DNA present in milk, meat and eggs?
- What studies have been conducted?

Regulatory Science and Safety of Biotech Crops

Comprehensive environmental, food and feed safety assessments are integral components of the product development and registration processes



Comprehensive safety assessments

Integrated (coordinated) framework involves three federal agencies to ensure biotech product safety

USDA: Environmental safety

EPA: Environmental, food and feed safety for pest-protected product registration and sales

FDA: Feed and food safety

Public participation is solicited by these agencies at several steps in the process, from small-scale testing to commercialization.

Comprehensive international safety assessments in key export markets

COUNTRY	AUTHORITY	TYPE OF APPROVAL
European Union	Dir 90/220:	Environment and Feed
	Novel Foods - Notification	Food
Canada	Health Canada	Food
	Canadian Food Inspection Agency	Feed and Environment
Japan	Ministry of Agriculture, Forestry and Fisheries	Feed and Environment
	Ministry of Health and Welfare	Food

These products have undergone a comprehensive and rigorous assessment process to validate their safety…

The safety of biotech products is established through the following approach

Gene / Protein Safety → **Food/Feed and Environmental Safety** ← **Crop Safety**

- **Gene(s)**
  - Source(s)
  - Molecular characterization
  - Insert / copy number / gene integrity
- **Protein(s)**
  - History of safe use and consumption
  - Function / specificity / mode of action
  - Levels
  - Toxicology / allergenicity testing
- **Crop Characteristics**
  - Morphology
  - Yield
- **Food/Feed Composition**
  - Proximate analysis
  - Key nutrients
  - Key anti-nutrients
- **Environmental Safety**

Safety of the Genes

- Detailed map of vector
- Identity of all genetic components of vector
- Portion and size of inserted sequences
- The function of the genetic component in the plant
- The source of the genetic component
- Inheritance and stability of trait

Safety of the Genes

- Is the genetic component/donor organism a pathogen?
- Does it produce a known toxicant, allergen, or irritant?
- Is there a history of safe use of the source organism?

Safety of the Proteins

- Indicate if there are changes in the amino acid sequence from the native protein.
- Submit data indicating if the protein is expressed as expected.
- Compare novel protein sequence to known toxins and allergens.
- Acute/Chronic testing, mouse.
- In vitro digestibility assay.

Comparison to toxins or allergens

- The protein is compared to proteins in large global databases
  - More than 100,000 different proteins are searched
- A "Macro" comparison looks at the whole protein
- A "Micro" comparison looks at small stretches of the protein
  - As few as 8 amino acids are compared
  - For the Cry proteins more than 600 searches are performed across the entire protein length

Toxicology testing

B.t. Cry1A(b) protein example

- 14 day acute mouse oral gavage, dose=**3283 mg/kg, no negative effect.**

- A 220 lb animal would need to consume over 70 tons of grain in one sitting to get the equivalent dose

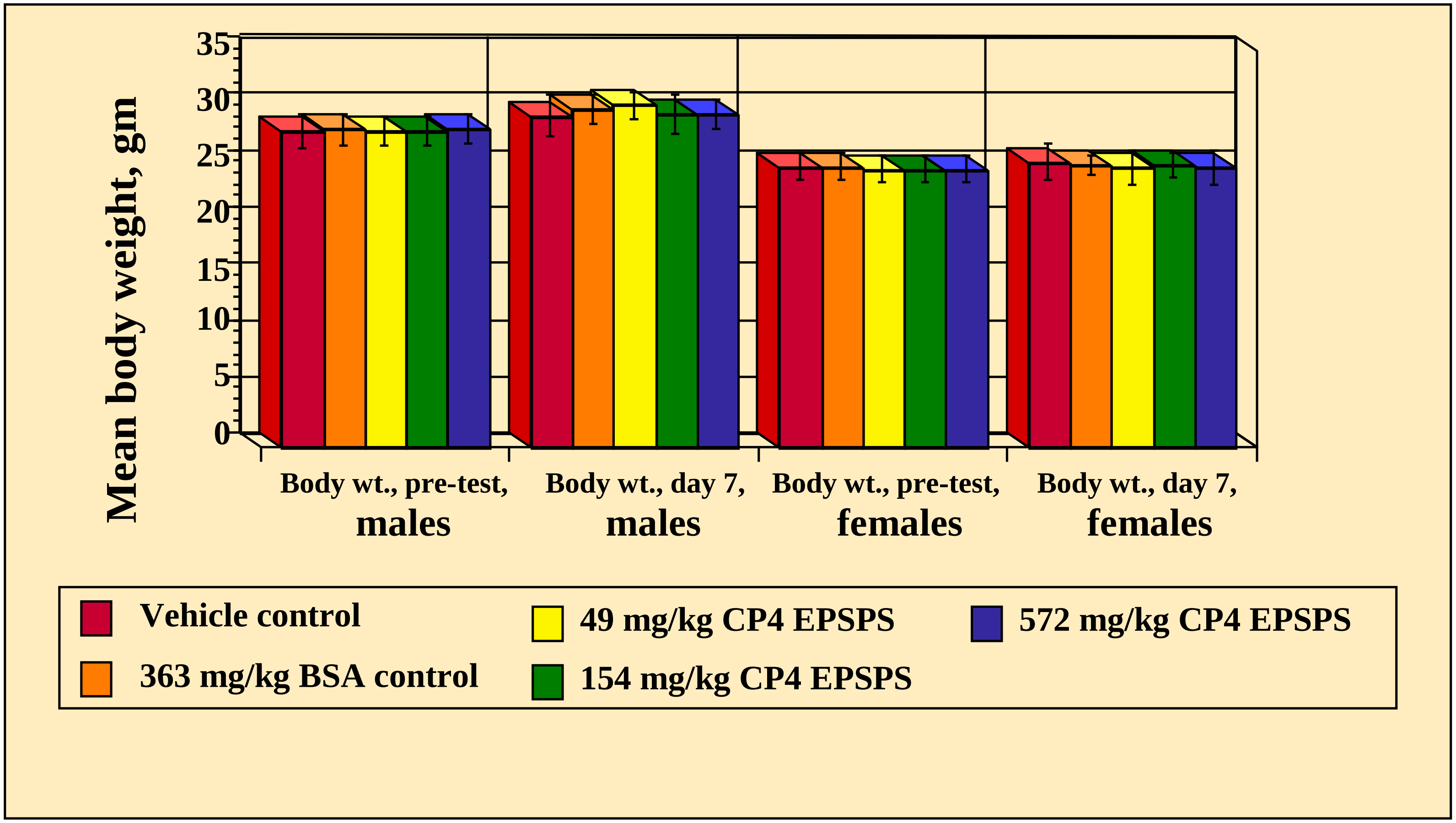
Toxicology testing results

Cry9C

- Mouse acute oral 3.3 g/kg **no effects**
  = 1 million × human daily intake
- Mouse 30-day oral 0, 33.3 & 328 mg/kg/day **no effects**
  = 2000 × human intake
- Mouse acute iv 0.3 mg/kg/day **no effects**
  = >1 billion × possible human dose

Toxicity Assessment: Roundup Ready/CP4 EPSPS protein

No deleterious effects at highest dose (572mg/kg)



Toxicity Assessment:

- Once protein safety established, EPA tests for all other secondary metabolic activities that result from protein expression
- e.g.: changing oil composition of canola from changing a protein
- These kinds of products would be labeled

Agronomic properties of biotech crops are thoroughly characterized:

- Evaluated in thousands of field trials in the United States, Europe, Canada and South America.
- Evaluated for a broad range of agronomic traits during the entire life cycle of the plant.
- Identical to their conventional counterpart except for the introduced trait(s).

Environmental Safety

- **Speed of soil degradation**
- **Impact on soil invertebrates**
  - Earthworms
- **Impact on aquatic invertebrates**
  - Daphnia
- **Impact on beneficial insects**
  - Ladybugs, parasitic wasps,honeybees and lacewings
- **Impact on fish**
  - Trout
- **Impact on birds**
  - Quail
- **Impact on mammals**
  - Mice
- **Insect resistance management**

Cry9C – StarLink™ Corn

- Bt protein from a different strain of *Bacillus thuringiensis*
- Deregulated by USDA
- Successful consultation with FDA
- Feed exemption granted by EPA
- Food tolerance exemption not yet finalized by EPA

Allergenicity Evaluations

True Food Allergies affect 1-2% of the adult population worldwide

"Big Eight" Major Food Allergens

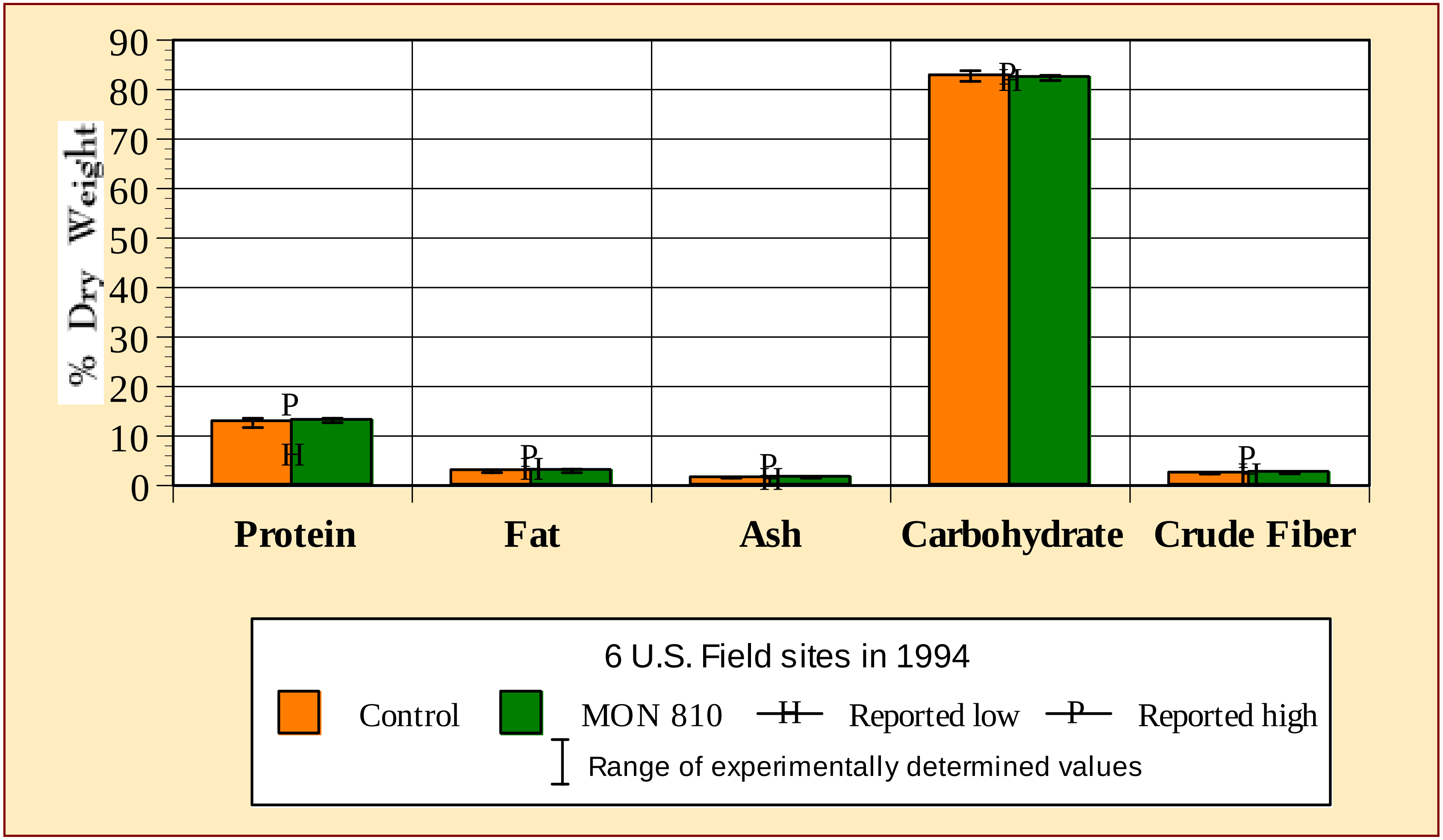
- Peanut
- Milk
- Soy
- Wheat
- Shellfish
- Fish
- Eggs
- Tree Nuts

Allergenicity Screen

Homology Search	**None w/known oral allergens**
Prevalence (1 – 10%) in Food	**< 0.3%**
Disulfide bonds	**None**
Heat stability	**Heat stable**
Digestible	**Not readily digested**

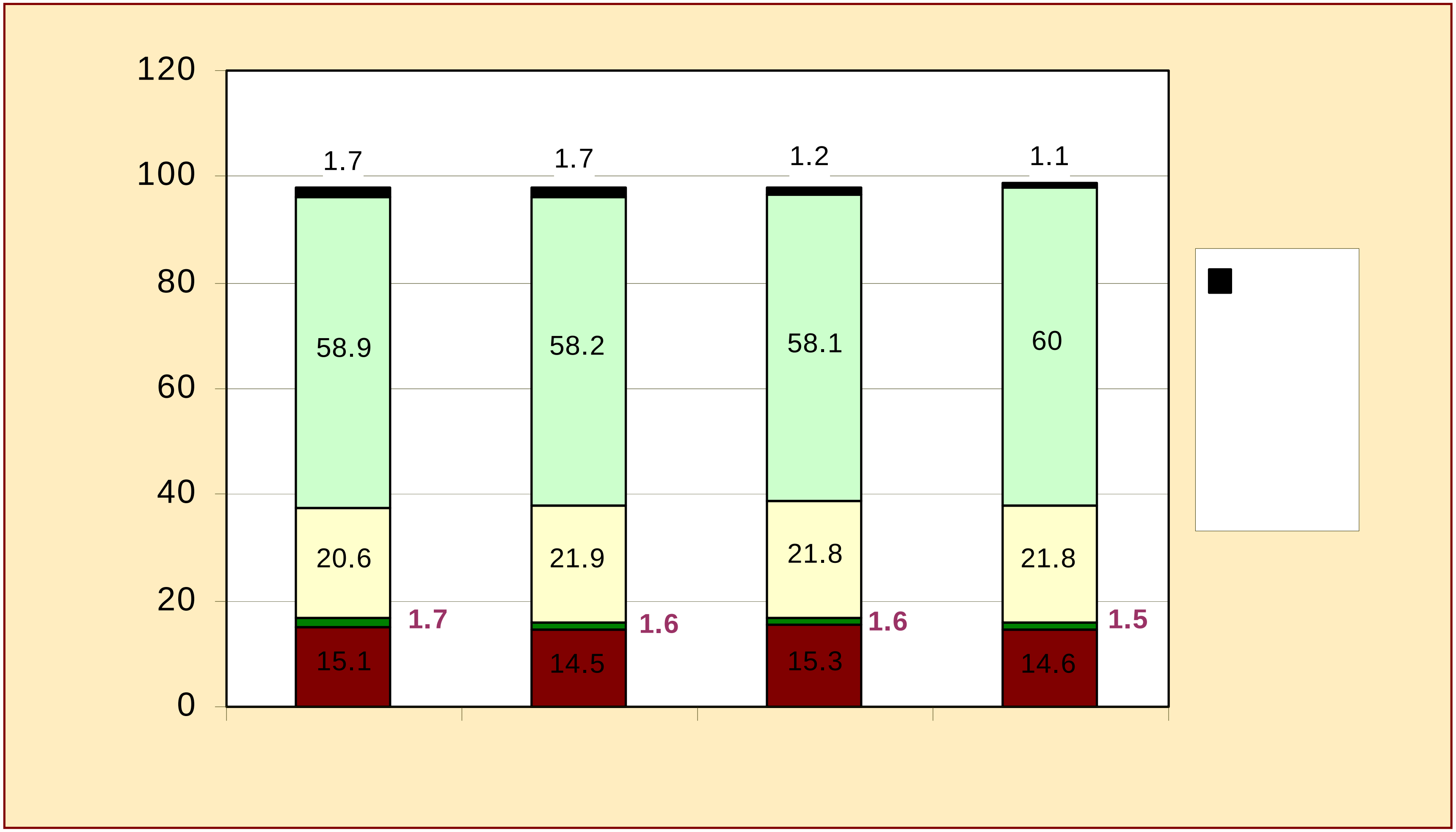
Compositional Equivalence...

Compositional Equivalence: Proximate Analyses

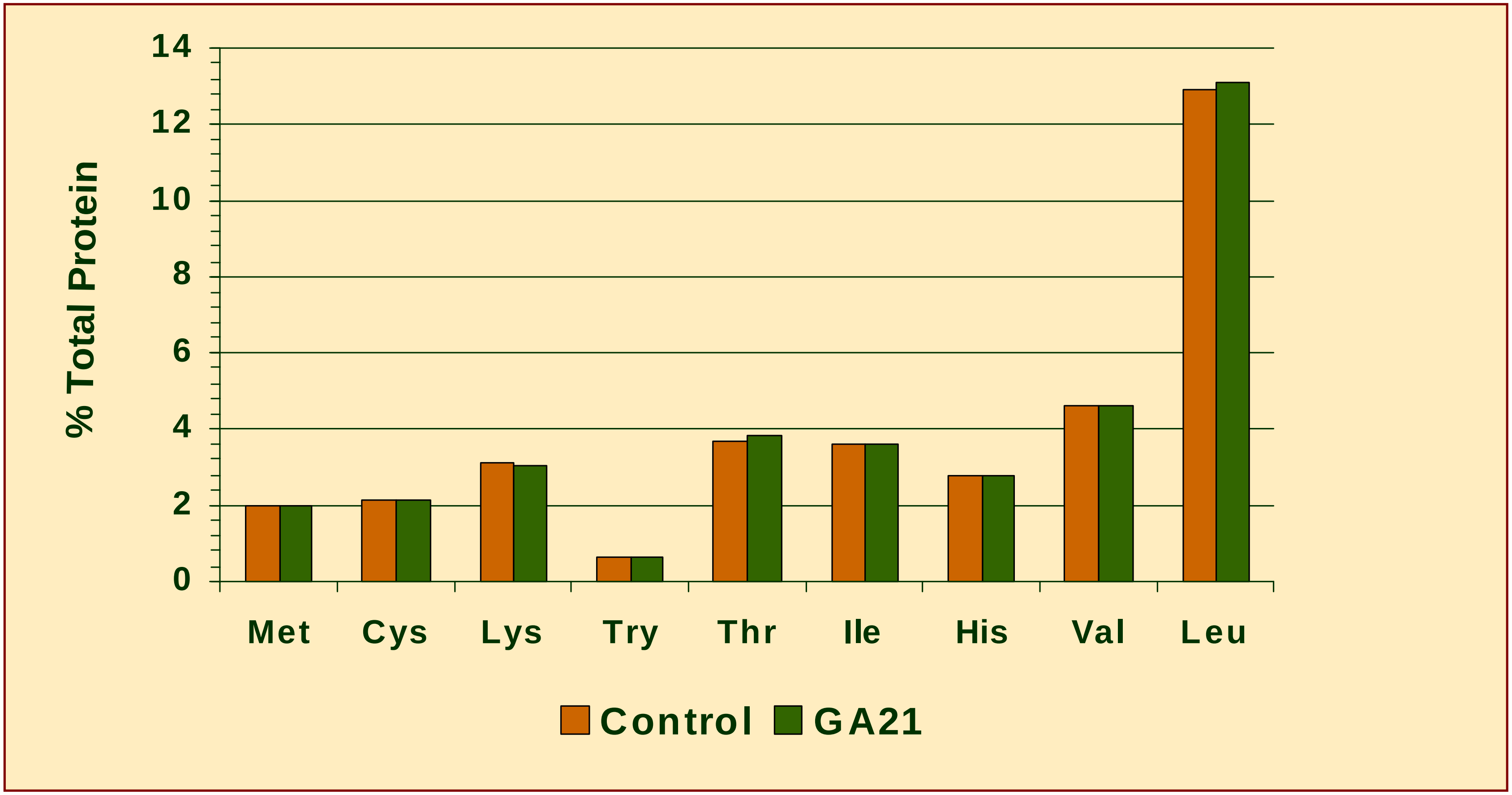


These results have been generated on Event MON810. Data showing similar proximate analyses have been generated on the other corn events.

Compositional Equivalence: Fatty Acids

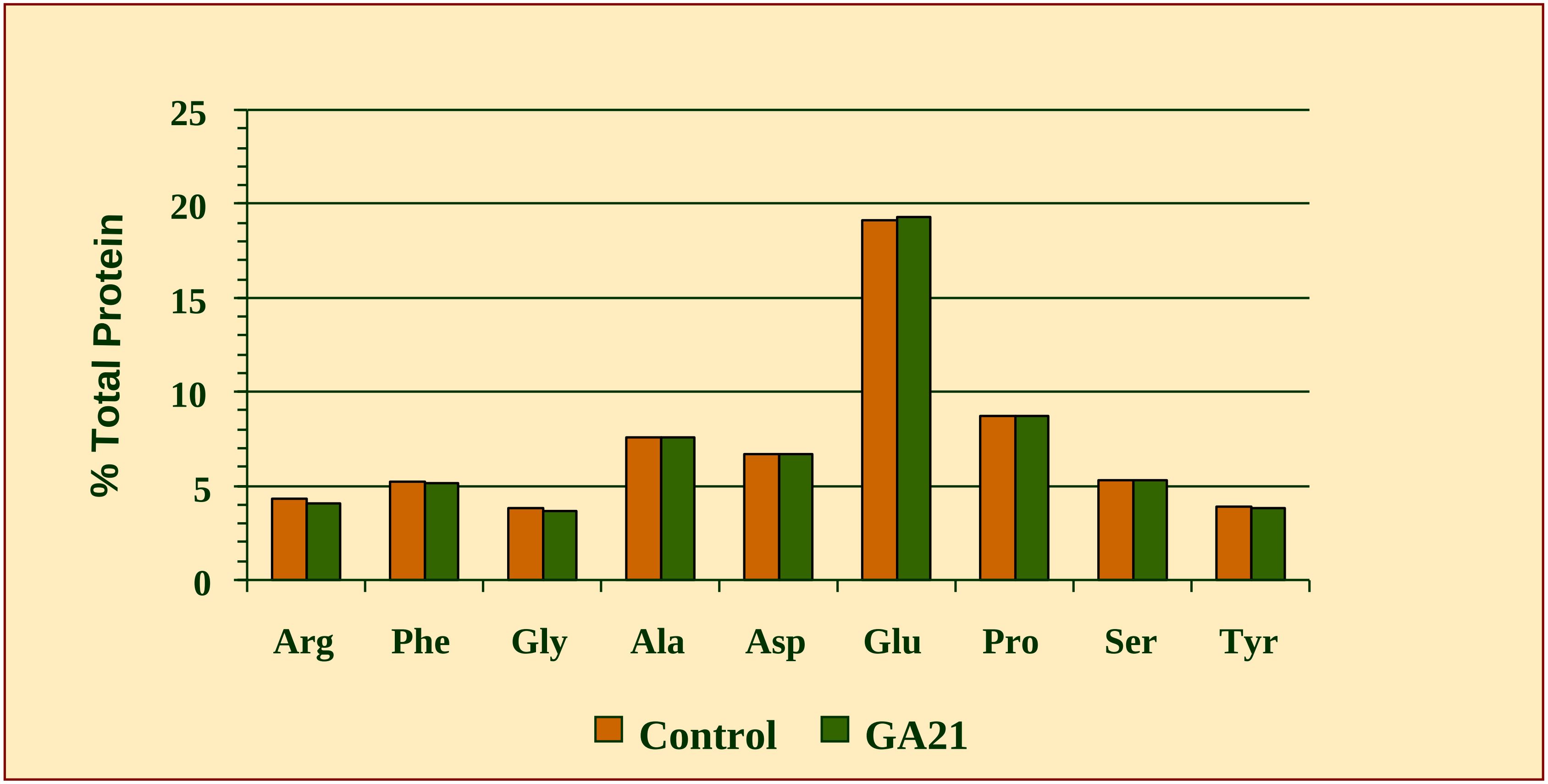


Compositional Equivalence: Amino Acids



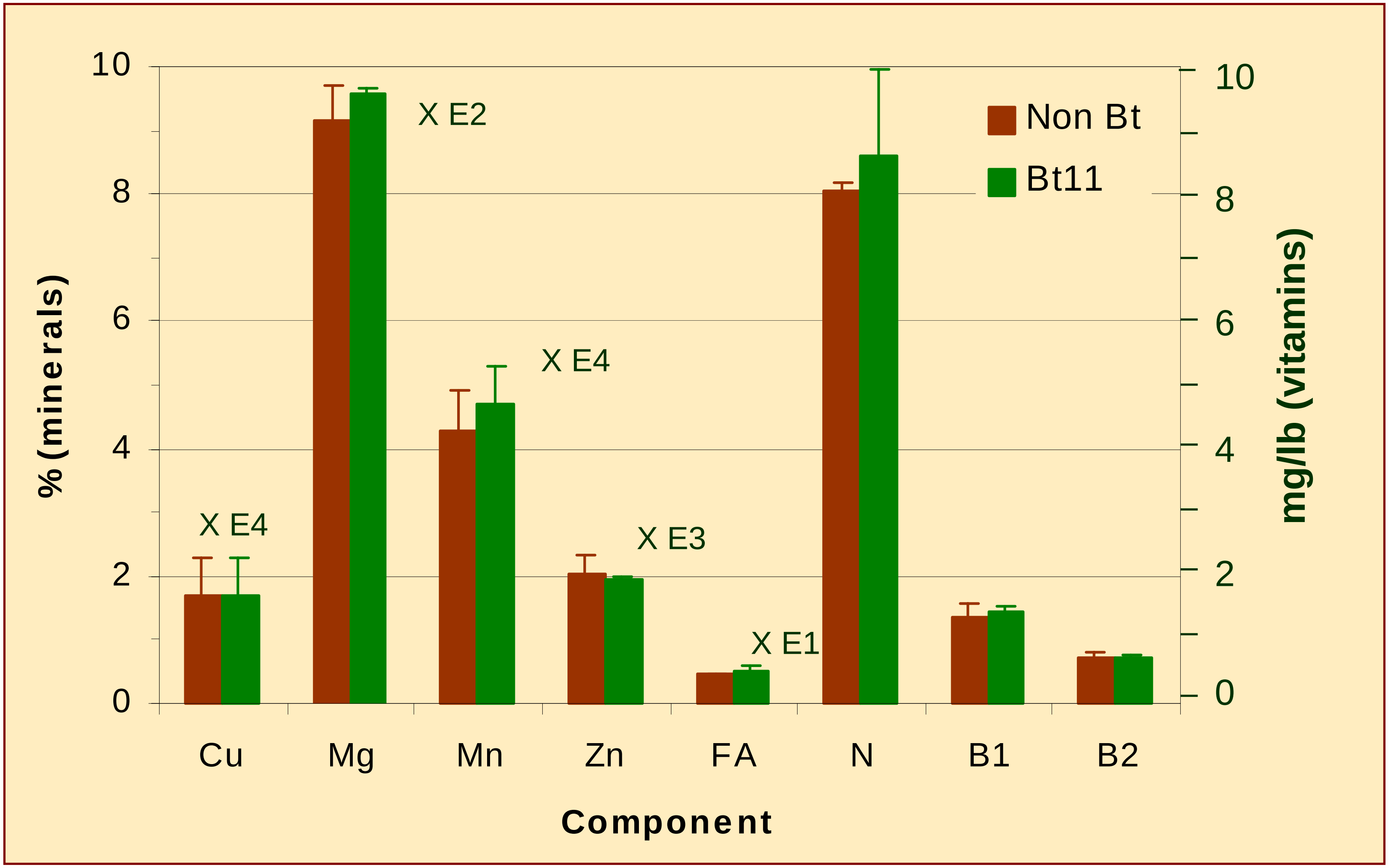
These results have been generated on event GA21. Data showing similar amino acid composition have been generated on the other corn events.

Compositional Equivalence: Amino Acids



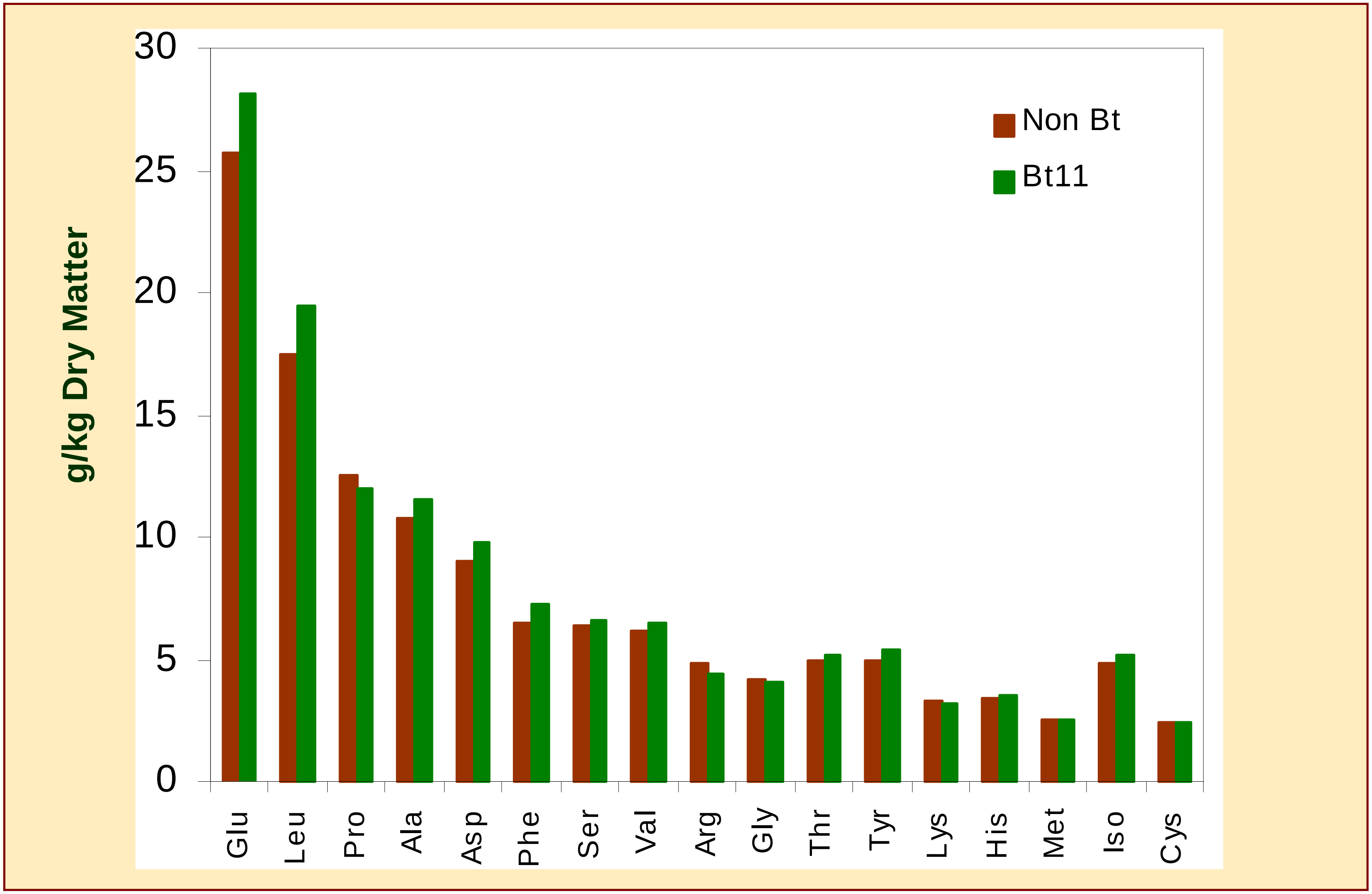
These results have been generated on event GA21. Data showing similar amino acid composition have been generated on the other corn events.

Compositional Equivalence: Mineral and Vitamin



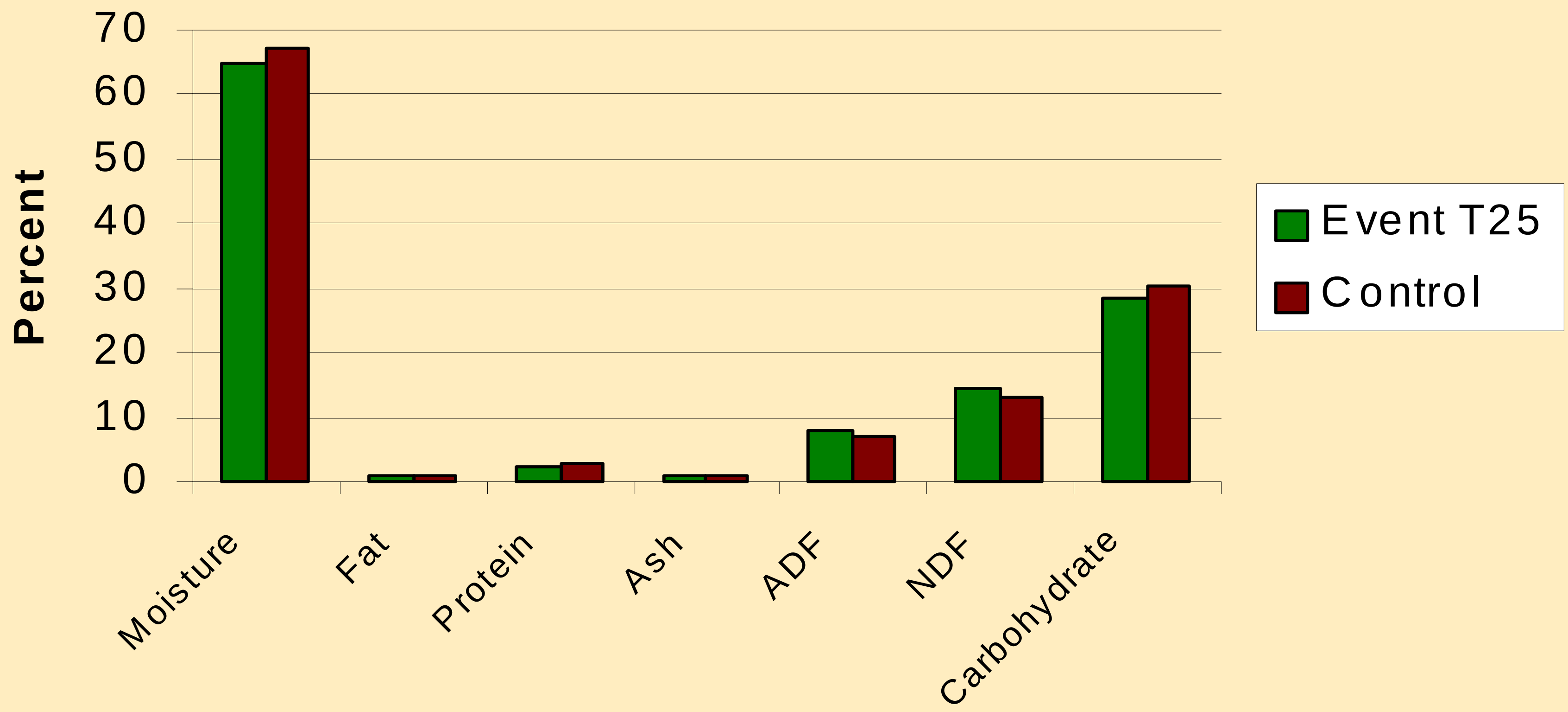
These results have been generated on event Bt11. Data showing similar mineral and vitamin composition have been generated on the other corn events.

Compositional Equivalence: Amino Acid



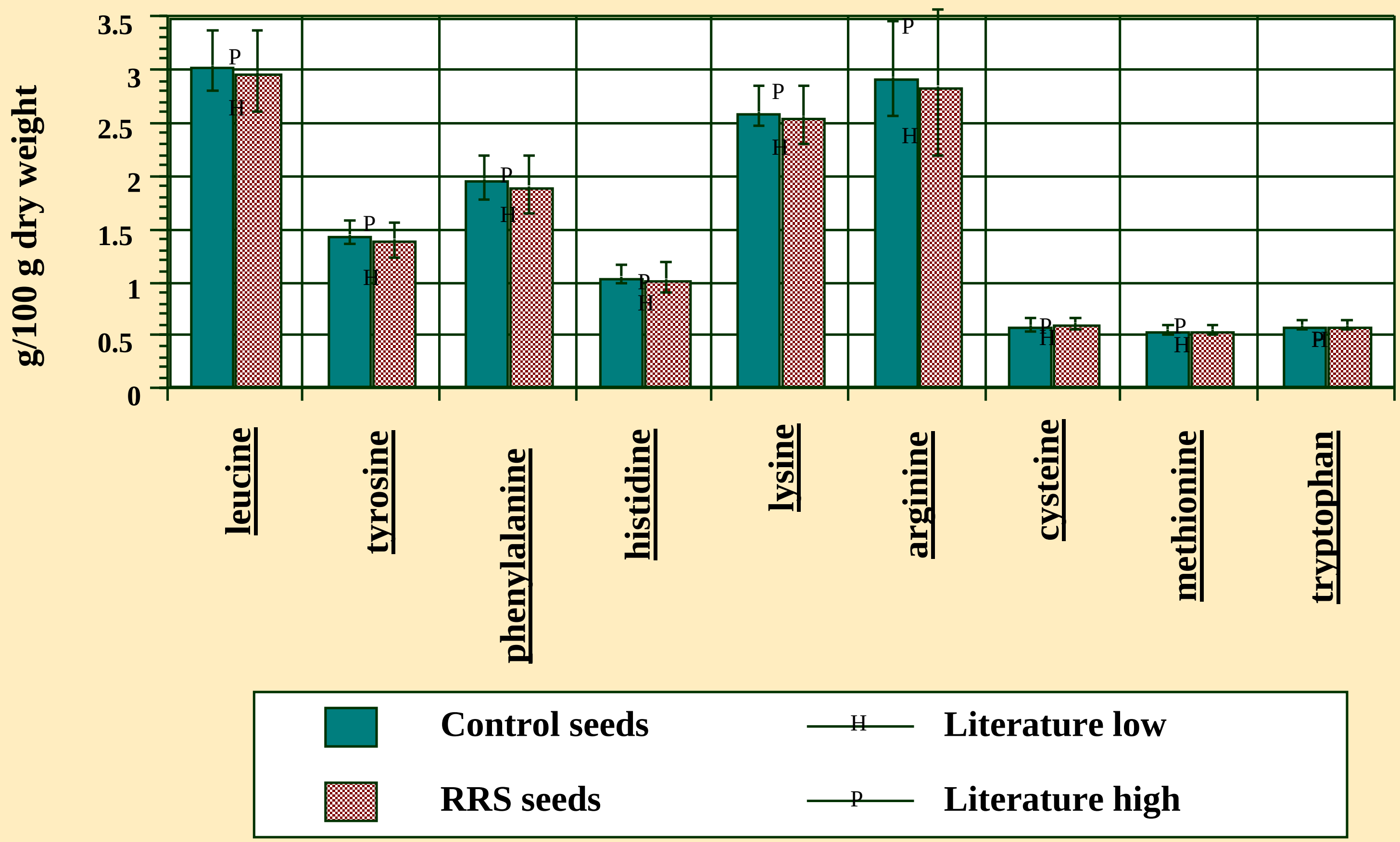
These results have been generated on event Bt11. Data showing similar amino acid composition have been generated on the other corn events.

Compositional Equivalence: Forage



These results have been generated on Event T25. Data showing similar forage composition have been generated on the other corn events.

Amino Acid Analysis of Seeds From Roundup Ready Soybeans

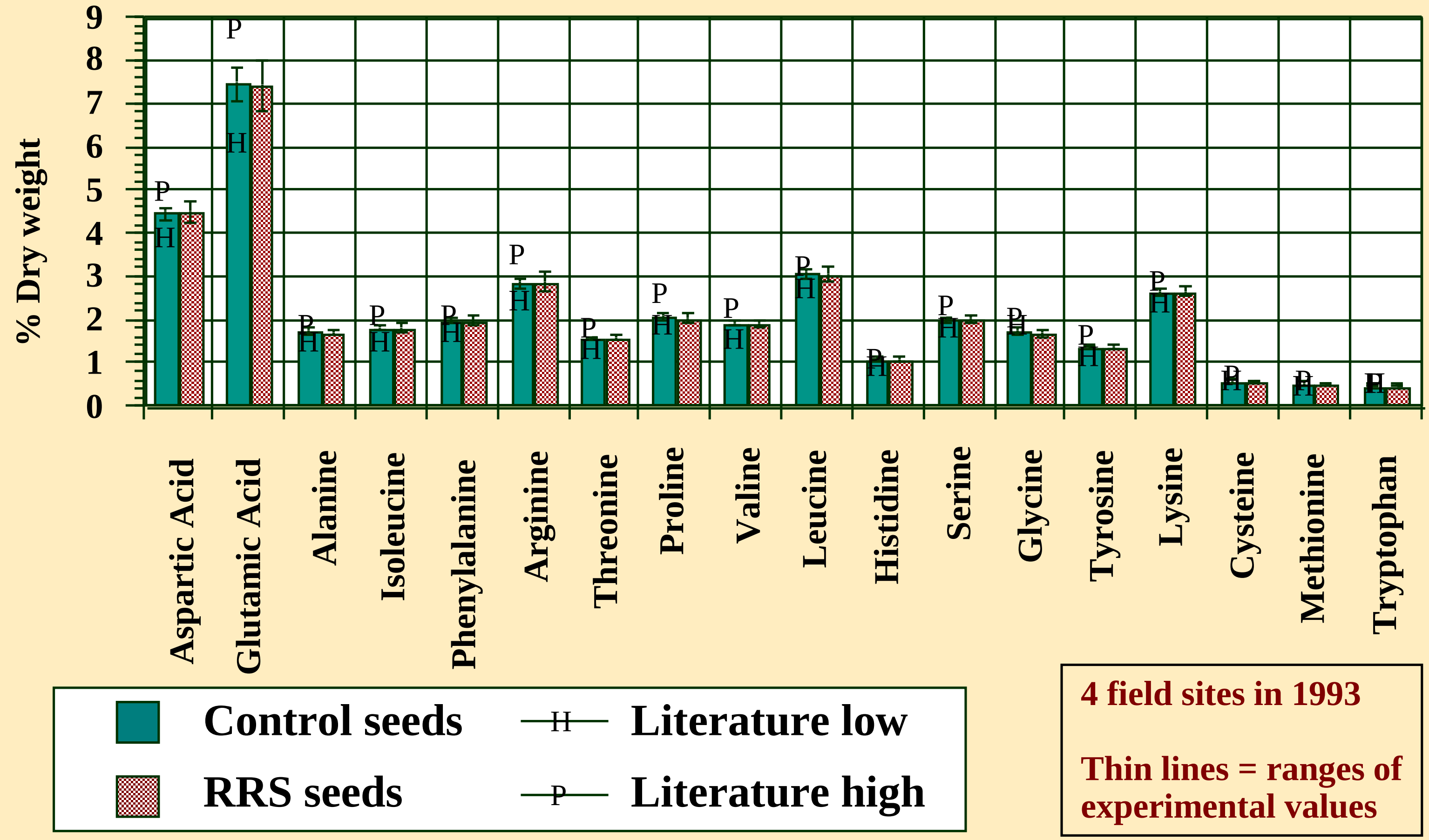


9 field sites in 1992

Thin lines = ranges of experimental values

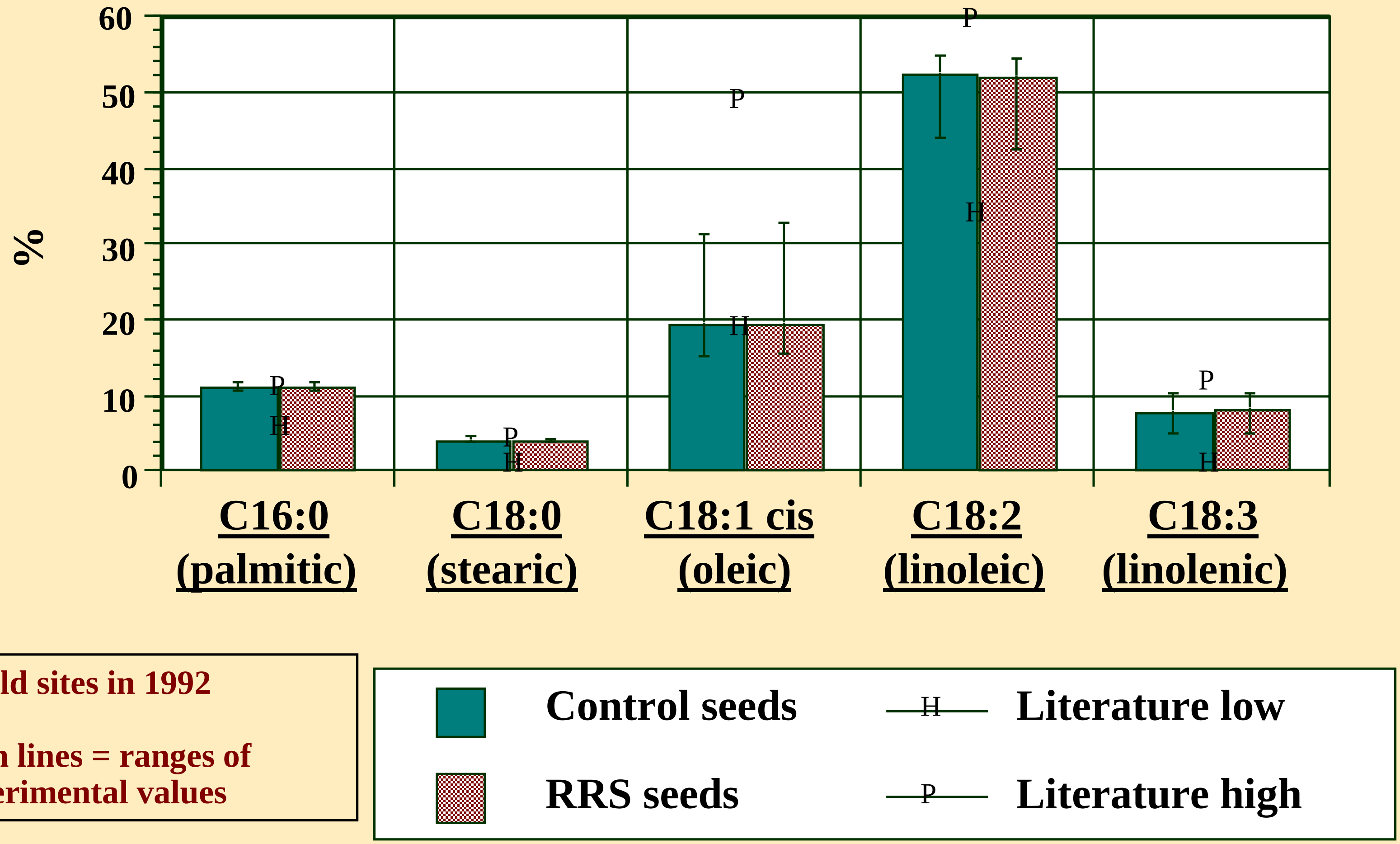
***No significant differences from the control line were observed at the 5% level (protected LSD).**

Amino Acid Analysis of Seeds From Treated Roundup Ready Soybeans



*No significant differences from the control line were observed at the 5% level (protected LSD).

Fatty Acid Analysis of Seeds From Roundup Ready Soybeans



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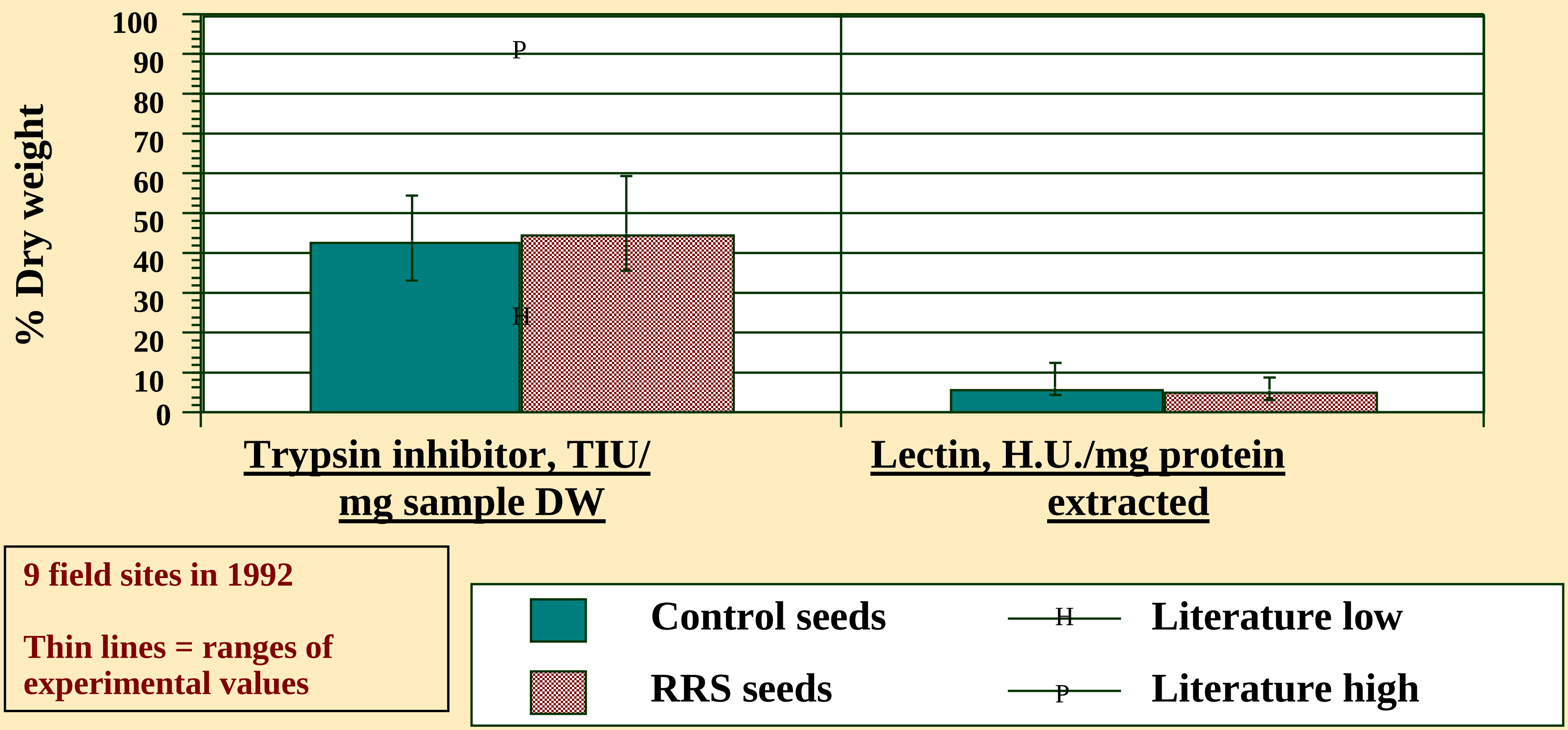
Antinutrients in Seeds From Roundup Ready Soybeans



*No significant differences from the control line were observed at the 5% level (protected LSD).

RR Soybeans are Compositionally Equivalent to Conventional Soybeans

Component	Beans	T Meal	Defat Flour	Isolate	Conc	RBDO
Proximate analysis	CE	CE	CE	CE	CE	
Amino acid comp	CE					
Fatty acid comp	CE					CE
Trypsin inhibitors	CE	CE	CE			
Lectins	CE	CE				
Phytoestrogens	CE	CE				
Urease	CE	CE	CE			
Stachyose, raffinose		CE				
Phytate		CE				
N-solubility		CE				

CE = Compositionally equivalent

- Based on assessment of over 400 components in 2000 independent analyses - J. Nutrition, 1996, 126:702-716
- Confirmed with soybeans treated with Roundup herbicide - J. Agri. Food Chem. (in press)
- Confirmatory macro analysis conducted in 1994, 1996 and 1998 (UK)
- No changes in endogenous soy allergens - J. Allergy Clin. Immunol. 1996, 96:1008-1010

Safety assessment summary for biotech crops

Component	Changed	Unchanged
Agronomics		🗐
Environmental safety		🗐
Composition		🗐
Additional gene(s) / protein(s)	🗐	
Introduced trait(s)	🗐	

These data establish the safety of these biotech products and fulfill international regulatory requirements

Animal Performance Data...

Feed Equivalence and Performance for Livestock

Purpose:

- Confirm nutritional equivalence in feeding studies with biotech crops compared to conventional counterparts.
- Demonstrate equivalence in performance.
- Demonstrate no unintended health effects.

Biotech Industry Approach to Confirm Nutritional Equivalence

- Demonstrate nutritional equivalency through performance trials
- Growing chick was chosen as the model of choice for early studies with corn and soybeans
  - Fast growing
  - Sensitive
  - APHIS requires destruction of test animals and materials until approval is granted
  - Limited available quantities of test grain
- Crops evaluated later with other species

42 animal feeding studies using biotech crops have been completed or are in progress

Results to date:

Animals perform in a comparable manner when fed biotech crops as compared to conventional counterparts...

Biotech product studies conducted by universities or scientific institutes

			Studies	
Trait	Crop	Animal	Completed	In Progress
H.T.	Corn	Chicken- broilers	2	-
H.T.	Corn	Beef cattle	1	1
H.T.	Corn	Swine	-	2
H.T.	SBM	Chicken-broilers	2	-
H.T.	SBM	Dairy Cows	1	-
H.T.	SBM	Swine	-	1
H.T.	SBM	Catfish	1	-
H.T.	Canola	Chickens - broilers	4	-
H.T.	Sugar beets	Sheep	2	1
H.T.	Sugar beets	Pigs	1	-

H.T.: Herbicide tolerance

Biotech product studies conducted by universities or scientific institutes

Trait	Crop	Animal	Studies: Completed	Studies: In Progress
B.t.	Corn	Chicken - broilers	5	1
B.t.	Corn	Chicken - layers	2	-
B.t.	Corn	Catfish	1	-
B.t.	Corn	Swine	-	2
B.t.	Forage	Sheep	1	-
B.t.	Forage/Corn	Dairy cows	1	4
B.t.	Forage/Corn	Beef cattle	1	5

B.t.: Insect protection

Feeding studies demonstrate nutritional equivalence

- ***Chicken studies***
  - **Demonstrated the safety and feed performance equivalence of Bt corn to conventional corn**

    Aulrich *et al*., 1998; and Halle *et al*. 1998 Institut für Tierernährung (Braunschweig, Germany)

    Brake and Vlachos, 1998 (NC State Univ. & Novartis)

    Mireles, Jr. *et al.*, 2000 (Foster Farms Feed Research)

Feeding studies demonstrate nutritional equivalence

- ***Beef steers grazing Bt or conventional corn stalks/residues***
  - **Reported no differences in performance or preference**

Folmer et al. (2000)
University of Nebraska

Feeding studies demonstrate nutritional equivalence

- **Beef cows grazing Bt or conventional corn stalks/residue**
  - **Reported no differences in performance in Year 1 and Year 2 of this two year study**

Russell & Peterson (1999)
Iowa State University

Russell et al. (2000)
Iowa State University

Hendrix et al. (2000)
Purdue University

Feeding studies demonstrate nutritional equivalence

- ***Feedlot cattle fed Bt and conventional corn silage***
  - **No differences in performance**

Folmer *et al.*, 2000 (University of Nebraska)

Hendrix *et al.*, 2000 (Purdue University)

Feeding studies demonstrate nutritional equivalence

- ***Lactating dairy cows fed Bt or conventional green chop***
  - **Reported no differences in feed intake, milk yield, milk composition or udder health**

Faust and Miller (1997)

Iowa State University

Feeding studies demonstrate nutritional equivalence

- ***Lactating dairy cows fed Bt or conventional corn silage and corn grain***
  - **Reported no differences in feed intake, milk yield, milk composition, efficiency, ruminal pH, acetate to propionate ratio or in situ NDF digestion kinetics**

Folmer et al. (2000)

University of Nebraska

Feeding studies demonstrate nutritional equivalence

- ***Sheep fed Bt or conventional corn silage***
  - **Reported no differences in digestibility in sheep**

Daenicke et al. (1999)

Institut für Tierernährung (Braunschweig)

Feeding studies demonstrate nutritional equivalence

- ***Growing bull calves fed Bt or conventional corn silage***
  - **Reported no differences in silage intake, body weight gain, feed conversion, hot carcass weight, dressing percentage and abdominal fat in bull calves slaughtered at 1180 lbs.**

Daenicke et al. (1999)

Institut für Tierernährung (Braunschweig)

Feeding studies demonstrate nutritional equivalence

- ***In vitro silage study***
  - **Demonstrated equivalence in digestibility and nutrient composition of Bt corn silage to conventional corn silage**

Faust (1997, 1999)

Iowa State University

Feeding studies demonstrate nutritional equivalence

- ***Lactating dairy cows fed Roundup Ready or conventional corn and corn silage***
  - **Reported no differences in feed intake, milk yield and milk composition.**

Donkin et al. (2000)

Purdue University

Feeding studies demonstrate nutritional equivalence

- ***Broilers fed Roundup Ready or conventional corn***
  - **Reported no differences in feed performance (feed intake, gain, feed efficiency and % fat pad)**

Sidhu et al. (2000)

Monsanto Company

Colorado Quality Research

Feeding studies demonstrate nutritional equivalence

- ***Broilers, catfish and dairy cows fed herbicide tolerant (H.T.) soybean meal or soybeans vs its conventional counterpart***
  - **Broilers: No differences in feed intake, gain, feed efficiency or breast meat and fat pad**
  - **Catfish: No differences in gain, feed efficiency or meat composition**
  - **Dairy cows: No differences in feed intake, milk yield, milk composition, digestibility, and rumen fermentation endproducts**

Hammond et al., 1996
Monsanto Company

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Detection of Recombinant DNA and Protein in Animal Products

Recombinant DNA and proteins

- **Recombinant proteins have been shown to be safe through required and voluntary safety testing studies**
- **World Health Organization and the US Food and Drug Administration have concluded there is NO inherent risk in consuming DNA, including DNA from transgenic crops**
- **Testing for recombinant DNA and proteins in animal products is NOT seen as necessary by Regulatory Agencies but is more of a livestock industry and consumer issue**

DNA and Protein Digestion

- **DNA and proteins are a natural and abundant component of our diet**
- **There is no difference between the digestion of endogenous and recombinant DNA**
- **Both recombinant and natural plant proteins are subject to the same digestive process**
- **The normal digestive process rapidly breaks down DNA and most proteins into smaller molecules for absorption**
- **Digestibility under simulated gastric conditions is a component of the product safety assessment**

Safety Studies vs. Performance Studies

SAFETY has been established for ALL food and feed-approved introduced proteins.

- safety studies include high-dose oral gavage mouse feeding with purified protein

  actual dose = 3283 mg Bt protein/kg gave - NO ADVERSE EFFECTS

  hypothetical dose = a 25 g mouse must consume 16.4 kg of Bt grain in one setting for an equivalent exposure

- performance studies include livestock feeding trials with a complex food source to evaluate nutritional equivalence

  hypothetical dose = a 100 kg animal must consume > 65,000 kg of Bt grain in one setting for an equivalent exposure

Challenges of Detecting Transgenic DNA or Introduced Proteins in Animal Products

- **To date, transgenic plant DNA and proteins have not been detected in milk, meat, or eggs**
- **Their presence is not expected based on our understanding of digestion and absorption of DNA and proteins**
  - Example: >90% of Cry1Ab *Bt* is degraded within 2 minutes in simulated gastric test. Estimated half life of Cry1Ab *Bt* is < 30 seconds
- **Assay challenge - distinguishing between true detection and accidental contamination**
  - care to avoid sample contamination (false positive result)
  - complex matrix/tissue could hinder DNA/protein isolation (false negative result)

Presence of Transgenic Plant Proteins in Feedstuffs

Feedstuff	*Protein(s)*	*Detection*
Corn – fresh green chop[1]	Cry1A(b), PAT	detected, detected
Corn – silage[2]	Cry1A(b)	not detected
silage[3]	Cry1A(b)	detected
total mixed ration[1]	Cry1A(b), PAT	detected, detected
Corn – wet milled[3]	Cry1A(b)	not detected
dry milled[3]	Cry1A(b)	detected
pellet. feed[4]	Cry1A(b)	detected
Soybeans – raw[5]	EP4 EPSPS	detected
Soybeans – meal[5]	EP4 EPSPS	detected

[1]Faust and Vlachos. Unpublished data.
[2]Fearing et al., 1997. Molecular Breeding. 3:169-176.
[3]Novartis Seeds. 2000. Personal Communication.
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[5]Ash et al., 2000. Poultry Sci. Assoc. Annual Abstracts. Suppl. 1:26.

Presence of Transgenic Plant DNA in Feedstuffs

Feedstuff	*DNA status*
Fresh – linseed, wheat, ryegrass, soy, corn, rapeseed, wet beet pulp	intact
Silage – ryegrass, corn	intact
Expelled – linseed, rapeseed	degraded
Extracted – soy, rapeseed	degraded
Dried beet pulp	degraded
Corn gluten feed	degraded
Steam flaked - corn	degraded

[1] Forbes et al., 1998. Scientific Report No. 376 to the Ministry of Agriculture, Fisheries, and Food, United Kingdom.

Estimate of Ingested Plant Protein

Based on the sensitivity of current methods, detection of recombinant protein is UNLIKELY.

- Detection of Cry1A(b) *Bt* protein in pork
  - Daily intake of Bt protein = 0.014 g
    assumes ~ 2.7 kg of feed eaten daily by 100 kg animal, feed = 55% Bt maize
  - Theoretical accumulation of Bt protein in pork = 0.3 fg/mg
    (Castell etal.)
  - Limit of detection by immunoassay = 50,000 fg/mg

Estimate of Ingested Plant DNA Levels

Based on the sensitivity of current methods, detection of recombinant DNA is UNLIKELY.

- Detection of CryIA(b) DNA in beef
  - Daily intake of total DNA = ~ 600,000 µg
    assumes 24 kg of feed eaten daily by 600 kg animal, feed = 80% recombinant maize grain recombinant gene coding region ~3.5 kb, haploid corn genome 2.5 Gbp
  - Daily intake of Cry1A(b) DNA = ~ 1.3 µg **(Beaver etal.)**
  - Assuming ~0.1% absorption of recombinant DNA **(Doerfler etal.)**

    approximately 0.002 fg DNA / mg tissue
  - Limit of detection of by conventional PCR

    approximately 0.002 fg DNA / mg tissue

Detection Methods

- **Protein detection**: Bt (CryIA(b)) sandwich immunoassay
- **DNA detection**: conventional PCR

Transgene Detection:

- Bt11 construct specific (35S/adh intron:*CryIA(b)*)
- Event 176 construct specific (PEPC:*CryIA(b)*)

Controls:

- bovine actin gene (multi-copy)
- bovine interleukin-2 receptor gamma gene (single copy)
- maize zein gene (multi-copy)
- maize amylose-extender gene (single copy)

Animal Performance Studies: DNA and Protein Detection

Poultry Feeding Study [1]

- diet = 61% grain (Bt and non-Bt isoline tested)
- samples collected on day 38
- midgut samples (22) analyzed for presence of DNA
- NO RECOMBINANT OR PLANT DNA SEQUENCES OR Bt PROTEIN DETECTED

Dairy Cow Feeding Study [2]

- diet = >30% green chop (Bt and non-Bt isoline tested)
- milk samples collected daily
- non-Bt (80), Event 176 (67), and Bt11 (80) samples analyzed for presence of DNA and Bt protein
- NO RECOMBINANT OR PLANT DNA SEQUENCES OR Bt PROTEIN DETECTED

1 Brake and Vlachos, North Carolina State University and Novartis Seeds

2 Faust and Miller, Iowa State University and Novartis Seeds

Animal Performance Studies: DNA and Protein Detection

Beef and Dairy Cow Study [1]

- diet = 70% corn silage (Bt and non-Bt isoline tested)
- muscle (24), spleen (24), milk (11) analyzed for presence of DNA and Bt protein
- NO RECOMBINANT OR PLANT DNA SEQUENCES OR Bt PROTEIN DETECTED

Poultry Feeding Study [2]

- diet = 64% grain (Bt and non-Bt isoline tested)
- samples collected on day 14
- muscle (20), liver (10), eggs (10) analyzed for presence Bt protein
- NO Bt PROTEIN DETECTED

1 Folmer etal. University of Nebraska and Novartis Seeds

2 Wildlife International Limited and Novartis Seeds

Future Detection Studies

- Industry working group formed to focus on joint studies:

Agricultural Biotechnology Stewardship Technical Committee (ABSTC)

  - Aventis
  - Dow AgroSciences
  - DuPont Specialty Grains/Pioneer
  - Monsanto
  - Novartis

- Funding a large PCR study to be carried out by a commercial DNA testing lab

ABSTC DNA Detection Study: Collaboration with GENE-SCAN GmbH

- To be completed by the end of 2000
  - total of 233 samples
    - chicken muscle, pork muscle, beef muscle, milk, liver, kidney tissue
  - PCR analysis for 35S promoter and a high copy endogenous maize gene
  - samples will be coded and randomized by a third party
  - GeneScan results will be decoded by Washington University's clinical laboratory
  - results will be published in a scientific journal
- Additional studies may be conducted if warranted by the results of the 2000 studies

Summary

- **Plant DNA and Bt protein have NOT been detected in meat, milk and eggs to date.**
- **Approved recombinant products have passed and often exceeded the appropriate regulatory requirements.**
- **Animal performance studies have confirmed safety and nutritional equivalence of approved recombinant products.**
- **The Biotech industry acknowledges our customers desire for additional animal performance and animal product information.**

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