Composition Testing in the Safety Assessment of Foods from Genetically Modified Plants

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Conflict of Interest Statement

I am an employee of Corteva Agriscience™ Agriculture Division of DowDuPont™ (DuPont Pioneer) which is a company that produces and sells seeds that are genetically modified.

Composition and Safety Testing

Outline

- Scope
- Why is composition testing conducted?
- Methods
- Results
- Feeding studies
- Consideration of metabolomic profiling
- Conclusions

Composition and Safety Testing

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- **Scope**
- Why is composition testing conducted
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Scope

Food from Genetically Modified (GM) Plants: What Role for Metabolomics?

- Review of current methods of composition testing
- Would metabolomics improve the safety assessment for foods from GM plants?

Scope

Foods and feeds from GM crops

- Protein safety testing discussed elsewhere

Regulatory *testing* – not necessarily regulatory *requirements*

- Differ by agency and geography
- Likely to change

Not product specific

Composition and Safety Testing

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Why is composition testing conducted?

Substantial equivalence

- Starting point in safety assessment of foods from GM crops
- Not possible to demonstrate that any food is “absolutely safe”
- Comparison approach is based on demonstrating compositional similarity to non-GM comparator with history of safe use (HOSU)
  - Near isoline

OECD 1993

Why is composition testing conducted?

Issue to investigate

- Possibility for unintended compositional effects

But…

- Which components to measure?
- What about natural variability?
- Spontaneous or introduced changes to DNA from traditional breeding practices

Composition and Safety Testing

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Methods

Primary components

- Composition
- Expression
- Agronomics

CEA Trial

Field trial design

- Composition (more later)
  - GM and non-GM comparator (near isoline) from same field
  - Additional non-GM commercially available reference lines
  - 8 different geographic locations
  - Grain (for animal feeding studies) and forage
  - 4 replicates of each entry
  - Validated analytical methods
- Expression
  - Of newly expressed proteins where applicable
- Agronomic performance

CEA Trial

Compositional testing

- Conducted with individual events and stacked traits
- Foods are complex mixtures but we know the identity of many components within them
  - Concentration ranges at which they are normally present
- Validated analytical methods
- Databases with historic ranges available
- Account for >95% of crop composition by weight

Chassy, 2010

CEA Trial

Compositional testing

- Nutrients
- Antinutrients
- Secondary metabolites
- Toxicants
- Endogenous allergens (EFSA)

CEA Trial

Compositional testing

- **Nutrients**
- Antinutrients
- Secondary metabolites
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CEA Trial

Nutrients

- Proximates
  - Moisture
  - Ash
  - Carbohydrate
  - Protein
  - Fat
- Fiber
  - ADF and NDF
- These are standard composition variables for nutritional studies

CEA Trial

Minerals (corn)

- Calcium
- Phosphorus
- Copper
- Iron
- Magnesium
- Manganese
- Potassium
- Zinc

CEA Trial

Amino acids (corn)

- Alanine
- Arginine
- Aspartic acid
- Cysteine/cystine
- Glutamic acid
- Glycine
- Histidine
- Isoleucine
- Lysine
- Methionine
- Phenylalanine
- Proline
- Serine
- Threonine
- Tryptophan
- Tyrosine
- Valine

CEA Trial

Fatty acids (corn)

- Palmitic (16:0)
- Stearic (18:0)
- Oleic (18:1)
- Linoleic (18:2)
- Linolenic (18:3)
- Arachidic (20:0) and Arachidonic (20:4)
- Eicosenoic (20:1)
- Behenic (22:0)

CEA Trial

Vitamins – Corn

- Vitamin B1 (thiamin)
- Vitamin B2 (riboflavin)
- Vitamin B3 (niacin)
- Vitamin B5 (pantothenic acid)
- Vitamin B6 (pyridoxine)
- Vitamin B9 (folic acid)

CEA Trial

Compositional testing

- Nutrients
- **Antinutrients**
- Secondary metabolites
- Toxicants
- Endogenous allergens (EFSA)

CEA Trial

Antinutrients – Corn

- Trypsin inhibitor
- Phytic acid
- Protease inhibitor
- Raffinose

Antinutrients – Soy

- Trypsin inhibitor
- Lectin
- Oligosaccharides
  - Raffinose
  - Stachyose
- Phytic acid

CEA Trial

Compositional testing

- Nutrients
- Antinutrients
- **Secondary metabolites**
- Toxicants
- Endogenous allergens (EFSA)

CEA Trial

Secondary metabolites (corn)

- Furfural
- Ferulic acid
- p-Coumaric acid

CEA Trial

Compositional testing

- Nutrients
- Antinutrients
- Secondary metabolites
- **Toxicants**
- Endogenous allergens (EFSA)

CEA Trial

Toxicants

- “Key” if known to exist
- None identified for soy and corn
- Examples:
  - Potato → Glycoalkaloids (solanine)
  - Canola → Erucic acid
  - Tomato → Steroidal glycoalkaloids (α-tomatine, dehydrotomatine)
  - Wheat → DIBOA, DIMBOA

CEA Trial

Compositional testing

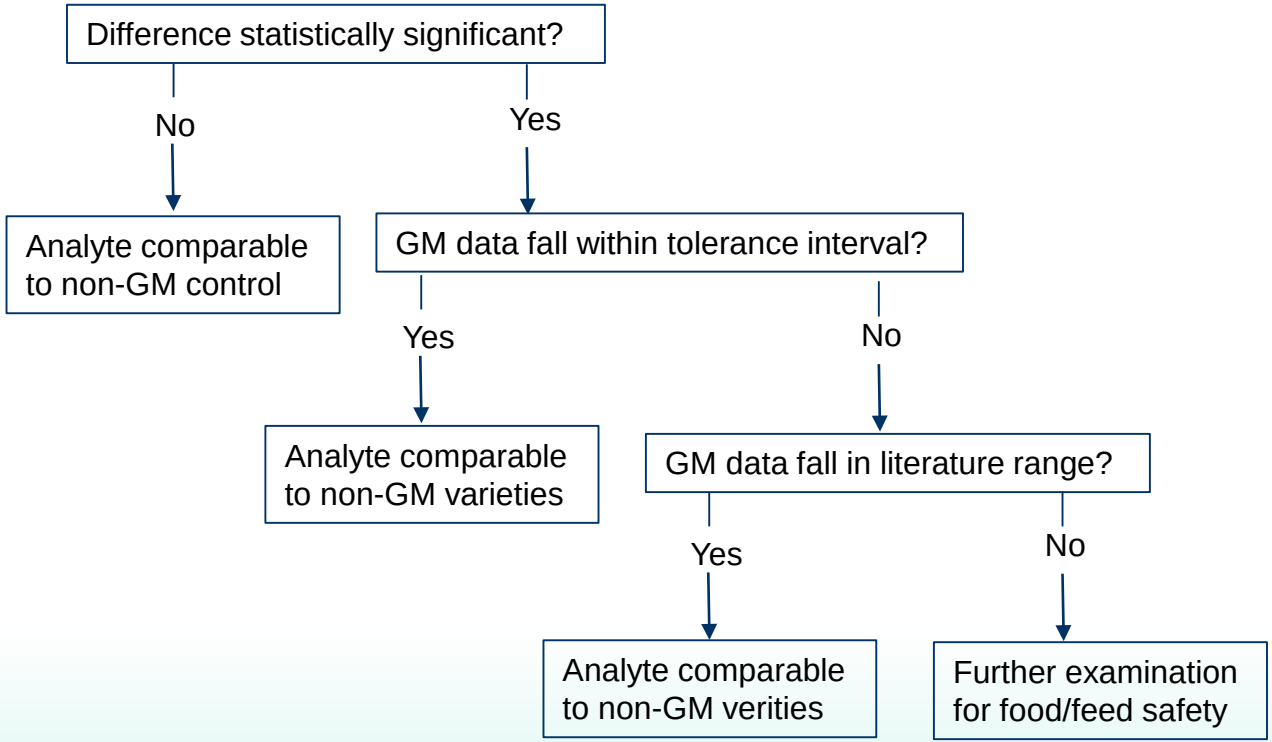
- Nutrients
- Antinutrients
- Secondary metabolites
- Toxicants
- **Endogenous allergens (EFSA)**

CEA Trial

Endogenous allergens (EFSA)

- Soybean
  - Gly m 1, 3, 4, 5, 6, 7, 8
  - Kunitz trypsin inhibitor
  - Gly m Bd 30K
  - Gly m Bd 28K
- Corn (Taiwan)
  - LTP

Statistical Analysis



Composition analysis

Has been conducted on numerous crops

- https://www.cropcomposition.org/query/index.html
  - Canola
  - Corn
  - Potato
  - Soybean
  - Cotton
  - Rice
  - Sorghum

Composition analysis

OECD Documents

- Corn
  - https://www.oecd.org/env/ehs/biotrack/46815196.pdf
- Soybean
  - https://www.oecd.org/env/ehs/biotrack/46815135.pdf
- Others
  - https://www.oecd.org

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Results

Environment and genetics have a greater impact on composition than biotechnology

- Cellini et al., 2004
- Reynolds et al., 2005
- Harrigan et al., 2010
- Herman and Price, 2013
- Harrison et al., 2013

Results

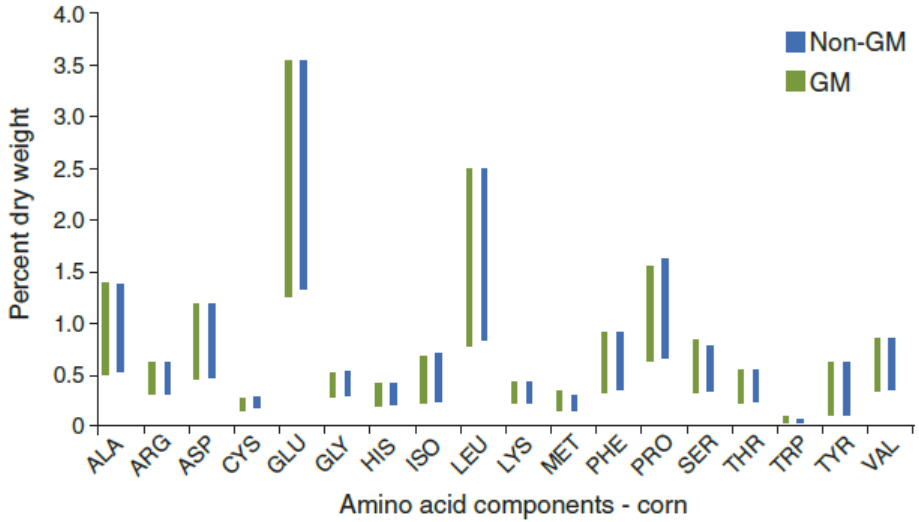

Figure 1 Summary of amino acid levels in conventional and GM corn from a total of eight growing seasons. Each vertical bar represents the range of values for the corresponding amino acids as measured in studies listed in **Supplementary Table 1**. See **Supplementary Table 20** for further details and **Supplementary Figures 1–11** for summarized data on other nutrient and antinutrient components in corn and soybean.

Harrigan et al., 2010

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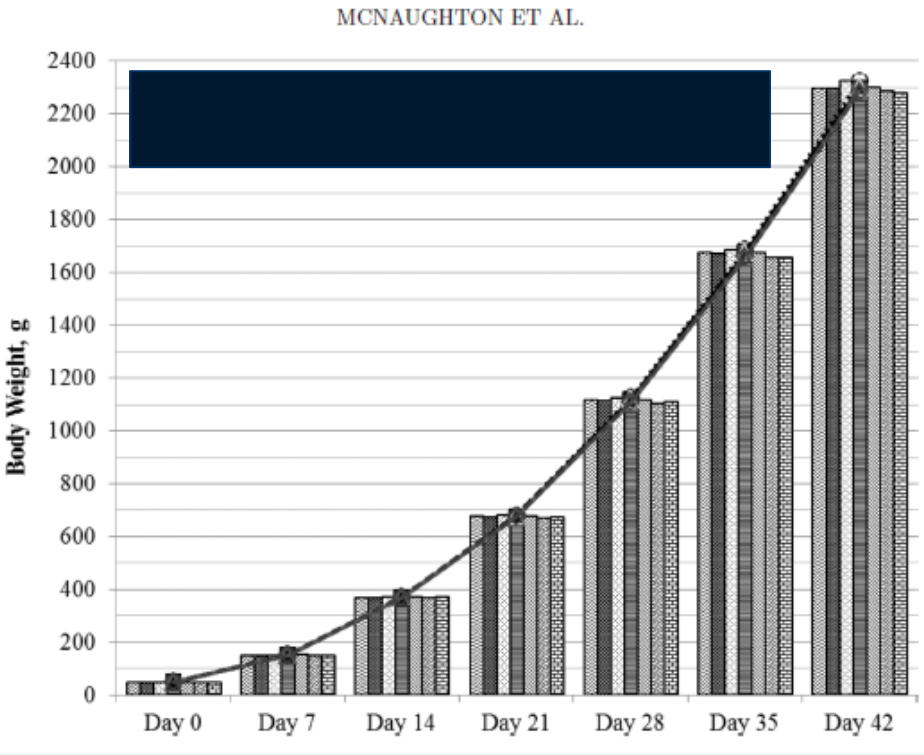
Feeding Studies

No adverse effects from animals consuming diets with foods from GM crops

- Conducted for comparison purposes
- Safety
  - Rat subchronic
- Nutritional comparison
  - Broiler chicken
  - Laying hen
  - Dairy cattle
  - Beef cattle
  - Swine

Feeding Studies



McNaughton et al., 2014

Feeding Studies

Results

- To date, no evidence of adverse nutritional effects from consumption of feed fractions from GM crops

Prevalence and impacts of genetically engineered feedstuffs on livestock populations[1]

A. L. Van Eenennaam[2] and A. E. Young

Department of Animal Science, University of California, Davis 95616

J. Anim.Sci. 2014.92:4255-4278
Doi:10.2527/jas2014-8124

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Consideration of Metabolomic Profiling

What is it?

- Analytical technologies to identify constituent components not typically evaluated in standard compositional analysis
  - Non-targeted
  - Not quantitative
  - Potentially hundreds or thousands of discrete substances
  - Present at small concentrations
  - Collectively <5% of total composition by weight

Consideration of Metabolomic Profiling

Has been conducted on GM crops

- Corn
  - Asiago et al., 2012
- Soybeans
  - Clarke et al., 2013

Consideration of Metabolomic Profiling

Results

- Changes in concentrations of substances not typically evaluated during composition analysis
  - Increase in some and decrease in others
  - Biological relevance?
  - Overall impact of biotechnology on composition was demonstrably lower in magnitude compared with environmental influence or when compared with genetic differences in crop varieties

Consideration of Metabolomic Profiling

Questions

- Can it increase knowledge about composition of foods from GM crops?
  - Yes
- Does it help in investigating the safety of foods from GM crops?
  - Yes/No/Maybe → Unclear
- Uncertainty about composition from analytical data?

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Conclusions

Composition testing of foods from GM crops:

- Analytical comparison of GM and non-GM products
- Substantial equivalence
- Genotype and environmental factors have more impact than GM

Conclusions

Omics profiling of GM crops could increase information about their composition but will not necessarily improve confidence in the safety assessment process

- Is more data necessarily better?

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