Comparative Assessment Studies:
Design and Interpretation of Results

GM food and feed safety assessment:
Training workshop for regulators
7-8 August, 2014

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Syngenta Crop Protection, LLC.
Compositional Data Analysis in the Safety Assessment of GM Crops

- Introduction to the comparative assessment: compositional analysis
- Choosing components to be assessed as they pertain to safety
- ILSI Crop Composition Database
- Design of field studies to control variability
- Interpreting compositional analysis data
Global area of GM crops: 1996 to 2012

27 countries growing GM crops
8 industrial
19 developing

World-wide adaptation of biotech crops

- 100-fold increase in area planted to GM crops since 1996 (1.7 million ha in 1996 vs. 175 million ha in 2013)

- Biotech crops fastest adopted crop technology in recent history – it delivers benefits (James, 2012)
Damage caused by *Diabrotica* spp. (corn rootworm)

- Non-GM control (susceptible)
- GM maize (resistant)
Brazil - Beans Resistant to Golden Mosaic Virus

Yield lost because of the virus would be able to feed 5-10 million people.

Photos: Francisco Aragão, EMBRAPA
Golden Rice: biofortified with beta-carotene

www.goldenrice.org
Many countries require comprehensive safety assessment of the GM crop

- General guidelines for the safety assessment have been laid out by the Codex Alimentarius Commission in 2003 (established by FAO/WHO)

- Regulations vary by country or region, although the *general* approach to the safety assessment remains fairly consistent with Codex

- Requirements for the safety assessment continue to evolve; crop composition continues to be an important part of the assessment
Comparative assessment contributes to the overall safety assessment.
Goal of Comparative Assessment: Composition

- Document nutritional status of the novel crop: as nutritious as the conventional crop having a history of safe use
  - Assesses the nutrition of the new GM crop and puts it in the context of the natural variability within the conventional crop

- Check for evidence of and document changes / effects:
  - **Intended effects** - product or activity of the transgene
  - **Unintended effects** - insertion of DNA or product(s) of transgenes could cause a change
  - Many of the nutritional components that are measured are end products of metabolic processes or are involved in these processes
  - Also investigated through molecular and agronomic studies
How to decide what Components to measure?
How to decide what Components to measure?

- Depends upon the crop
  - Valuable starting point - Individual crop consensus documents prepared by the Organisation for Economic and Co-operative Development (OECD)
  - Levels of key nutrients, anti-nutrients, toxicants, appropriate secondary metabolites (based on contributions the crop makes to the diet)

- Analysis should be on raw agricultural commodity

- Depends upon the nature of the trait(s) of interest
  - Some types of traits may directly influence metabolism (example: traits for stress tolerance)
  - Decisions on what to include should be hypothesis-driven
Experimental phase: Nutritional equivalence of GM crop to non-transgenic crop with history of safe use

- Replicated field trials at multiple locations
- Sample plant tissues relevant to food and feed
- Analyze samples for key nutritional & anti-nutritional components
- Compare GM crop to non-GM comparator (control)
New GM Crop Composition data

Statistically Different than Comparator?  
  Yes → Further investigate observed differences
  No → As safe as conventional crop

Comparative Assessment: Interpretation of Results

Further investigate observed differences

Compare with variability in:
  Comparator
  Conventional crop varieties
  Literature values, databases

Part of overall Safety Assessment
Assessing Biological Relevance

“The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance.”


Statistical significance ≠ Biological relevance
Sources of Variation in Crop Composition

- Experimental Design

- Experimental Conduct
Sources of Variation in Crop Composition

● Experimental Design
  - Lack of proper treatment randomization
  - Improper placement of blocks (replicates) in the field
  - Plot size / number of plants
  - Not enough locations to capture environmental effects

● Experimental Conduct
  - Plots not all treated the same (except for the intended treatments)
  - Failure to control known (control-able) environmental factors
    • Examples: non-uniform weed or insect pressure, movement causing soil compaction
  - Bias in sampling / data collection methods
Sources of Variation in Crop Composition

Fe content in common bean

Genotype
Sources of Variation in Crop Composition

Protein content in soy across a field

Environment
Sources of Variation in Crop Composition

Fe content in common bean

Protein content in soy across a field

Genotype x Environment

Genotypes differ in their response to various environmental factors.
Variability in a crop is expected and desirable

- Genetic background of a crop is diverse, and the food we eat on a daily basis is highly variable.

- Crop breeders actively search out diversity.

- Traditional breeding methods, used safely for thousands of years, can cause intended and unintended changes in genetics and composition.

- Amount of variability depends on the specific crop component.
Variability in a crop is expected and desirable

- Some components do not act independently
  - Example: protein and starch tend to be negatively correlated

- **Crop variability captured in:**
  - the control genotype within the study
  - reference varieties within the study
  - literature
  - private and public data bases
Role of Crop Composition Databases

- These data are important tools in helping to describe the boundaries of natural variability for each crop (considered to be safe levels)
- Easily-accessed and *updatable* compilations of crop composition data
- Help to capture the variability of component levels across broad geographies, multiple environments, and multiple years

From: Blair, ILSI CCW, 2012
Extracting Data From the Database

How to access the ILSI Crop Composition Database (CCDB) and how to navigate through the different screens

www.cropcomposition.org
Welcome to the ILSI Crop Composition Database

Version 4

The ILSI Crop Composition Database is a project of the International Life Sciences Institute.

The first three versions of the database have represented a compilation of crop analyses from a number of companies engaged in agricultural life sciences. Through ILSI, the participants have standardized and pooled their crop data in order to make the data available to scientists from academia, government agencies, and industry, and to the general public.

It is envisioned that future versions of the database will include other publicly available data that meet the acceptability criteria of ILSI and are submitted from scientists and other researchers representing a variety of public and private organizations.

NOTE: With the Version 4 upgrade, the system has been completely redesigned for increased speed and efficiency. No additional data were incorporated into the database from Version 3.0; however, minor differences in search results may be apparent due to the requirements associated with restructuring of the database. You will not be able to go back to Version 3.0 to replicate earlier search results now that Version 4 has been released.

Results from your search of Version 4 (the current version of the database) represent analyses of only conventionally bred crops.

- Database Search
- Register for Updates
- Other Web Sites of Interest
- About the ILSI-CCDB
- How to Cite this Database
- Frequently Asked Questions
- Help
Primary Search Criteria (Screen 2)

Search Crop Composition Database v4.2

Primary Search Criteria

The first step in searching the Crop Composition Database is to select your primary search criteria to filter the data sets.

You must select one Crop Type and one Tissue Type. You can further filter your results by optionally choosing one or more Crop Years, and Locations.

If you make no selections other than Crop Type and Tissue Type, all data sets for the chosen Crop-Tissue selection will be included.

Crop Source / Crop Type / Tissue Type

<table>
<thead>
<tr>
<th>Crop Type</th>
<th>Tissue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn - Field - Maize - Zea mays</td>
<td>Grain</td>
</tr>
</tbody>
</table>

Crop Year

- All Years
- 2005
- 2004
- 2003
- 2002

Location

<table>
<thead>
<tr>
<th>Country(s)</th>
<th>Region(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Countries</td>
<td>All Regions</td>
</tr>
<tr>
<td>ARGENTINA</td>
<td></td>
</tr>
<tr>
<td>AUSTRALIA</td>
<td></td>
</tr>
<tr>
<td>BRAZIL</td>
<td></td>
</tr>
<tr>
<td>BULGARIA</td>
<td></td>
</tr>
</tbody>
</table>

Analyte Filters (Optional)

View Summary of Search Results >

BY SUBMITTING SEARCH, YOU AGREE TO THE TERMS OF USE
Summary of Search Results (Screen 3)

Query Summary

The Query Summary shows the criteria that was used to filter the result set.

Summary of Search Results

The Summary of Search Results shows the results of your initial search grouped by the Analyte Types for the Data Sets that were found.

You can expand each Analyte Type to see the total number of samples and the number of samples with data below LOQ (denoted as X < LOQ) reported for each analyte. Expanding an Analyte Type also reports the minimum, maximum, and mean values for the samples in the primary unit of measure; these minimum, maximum, and mean values derive from data that is above LOQ for that analyte.

All analytes in the database have been assigned a primary unit of measure, which is shown in the right column of the new Summary of Search Results tool. If secondary units of measure (or multiple units of measure for a single analyte) are
## Summary of Search Results (Screen 3)

### Query Summary

The Query Summary shows the criteria that was used to filter the result set.

<table>
<thead>
<tr>
<th>Query Criteria</th>
<th>Help</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop Type:</td>
<td>Corn - Field - Maize - Zea mays</td>
</tr>
<tr>
<td>Tissue Type:</td>
<td>Grain</td>
</tr>
</tbody>
</table>

### Summary of Search Results

The Summary of Search Results shows the results of your initial search grouped by the Analyte Types for the Data Sets that were found.

You can expand each Analyte Type to see the total number of samples and the number of samples with data below LOQ (denoted as X < LOQ) reported for each analyte. Expanding an Analyte Type also reports the minimum, maximum, and mean values for the samples in the primary unit of measure; these minimum, maximum, and mean values derive from data that is above LOQ for that analyte.

All analytes in the database have been assigned a primary unit of measure, which is shown in the right column of the new Summary of Search Results tool. If secondary units of measure (or multiple units of measure for a single analyte) are preferred, Version 4.0 of the ILSI-CCDB requires that data with secondary units of measure be generated and viewed using an output report.

You can use this information below when defining the specific Analytes you would like to display in your final report.

LOQ = limit of quantitation, ND = not determined. In those cases where all measured values for a specific

### Results matching your query criteria

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Samples</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amino Acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Bio Actives</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fatty Acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fiber</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Minerals</td>
<td>Calcium</td>
<td>130 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>10.9</td>
<td>169.0</td>
<td>41.3</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Chloride</td>
<td>53 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>320.0</td>
<td>800.0</td>
<td>526.6</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>1211 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>0.64</td>
<td>14.78</td>
<td>1.55</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>1215 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>9.21</td>
<td>44.70</td>
<td>19.40</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>1217 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>534.6</td>
<td>1,726.6</td>
<td>1,067.9</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>1216 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>1.43</td>
<td>13.00</td>
<td>8.51</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
<td>1309 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>1,323.9</td>
<td>4,743.7</td>
<td>2,902.8</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>1217 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>1,529.0</td>
<td>5,368.7</td>
<td>3,422.0</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Selenium</td>
<td>184 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>0.05</td>
<td>0.69</td>
<td>0.19</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>1152 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>0.15</td>
<td>0.54</td>
<td>0.20</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Sulfur</td>
<td>53 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>454.0</td>
<td>1,170.0</td>
<td>744.1</td>
<td>ppm FW</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>1217 g&lt;sub&gt;LOQ&lt;/sub&gt;</td>
<td>5.6</td>
<td>34.3</td>
<td>19.2</td>
<td>ppm FW</td>
</tr>
<tr>
<td>Other Metabolites</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proximates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Summary of Search Results (Screen 3)

Create Report from Search Results

Choose the Analytes you would like to display in your report output.

You may choose individual Analytes or select an Analyte Type and choose to display all Analytes for the Analyte type chosen.

Once you have selected the analytes, click on the "add analyte(s)" button to confirm your selection.

To remove a selected analyte click on the red 'X' next to the analyte name.

Select Fields for Report Output.

In this section you can choose the metric fields and other fields you would like to display in your report.

More Below
### Summary of Search Results (Screen 3)

<table>
<thead>
<tr>
<th></th>
<th>Sulfur</th>
<th>5.3 (LOQ)</th>
<th>454.0</th>
<th>1,170.0</th>
<th>744.1</th>
<th>ppm FW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zinc</td>
<td>1217 (LOQ)</td>
<td>5.6</td>
<td>34.3</td>
<td>19.2</td>
<td>ppm FW</td>
</tr>
</tbody>
</table>

- Other Metabolites: not included
- Proximates: not included
- Vitamins: not included

### Analytes to show in output

**Analyte Type**: Minerals

**Analyte List**: All Minerals

**Units**: mg/kg DW

**Add Analyte(s)**

### Analyte List

**Metric Fields**

- Minimum Value
- Maximum Value
- Mean Value

- Number of Samples
- Samples Below LOQ
- Samples Above LOQ

More Below

---

**More Below**
Summary of Search Results (Screen 3)

Select Fields for Report Output.

In this section you can choose the metric fields and other fields you would like to display in your report.

Check the metric fields and grouping fields you would like to display in the output report.

Please note that selecting Seed Variety and/or Seed Vendor will disable the Detailed and Tabular Reports.

Metric Fields
- Minimum Value
- Maximum Value
- Mean Value
- Number of Samples
- Samples Below LOQ
- Samples Above LOQ

Grouping Fields
- Analyte Type
- Analyte
- Crop Year
- Crop Type
- Crop Source
- Tissue Type
- Seed Vendor
- Seed Variety
- Country
- Region
- Analysis Method

< Revise Query Filters  New Query  Report Options >
Report Options (Screen 4)

In this section you can choose the options that affect how the results of your search will be presented in the final report.

Start by choosing one of the predefined Report Types.

You must then choose an output format.

To print your report use the pull-down menu labeled Output Format to save a PDF to your desktop, and then print the PDF from your desktop.

And lastly, you can optionally provide a Title and Description to display at the top of your report and choose whether to display the Query Criteria and Report Options on your final report.

You may go back and forth between this page and the final report to refine your output.
Data: *Summary Report (Screen 5)*

Summary Report

### Report Output

**Output Format:** HTML

---

#### Mineral Levels in Maize

Search across all countries and years

Query Criteria:
- Crop Type is Corn - Field - Maize - Zea mays
- Tissue Type is Grain

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Minimum Value</th>
<th>Maximum Value</th>
<th>Mean Value</th>
<th>Number of Samples</th>
<th>Samples Below LOQ</th>
<th>Samples Above LOQ</th>
<th>Unit of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>47</td>
<td>47</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Calcium</td>
<td>12.7</td>
<td>208.4</td>
<td>46.5</td>
<td>1,309</td>
<td>5</td>
<td>1,304</td>
<td>mg/kg DW</td>
</tr>
</tbody>
</table>

[< Revise Report Options] [New Query]
## Data: Summary Report (Screen 5)

### CSV Report (comma-delimited text)

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Minimum Value</th>
<th>Maximum Value</th>
<th>Mean Value</th>
<th>Number of Samples</th>
<th>Samples Below LOQ</th>
<th>Unit of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>47</td>
<td>47</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Calcium</td>
<td>12.7</td>
<td>208.4</td>
<td>46.5</td>
<td>1,309</td>
<td>5</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Chloride</td>
<td>375.6</td>
<td>892.9</td>
<td>599.4</td>
<td>53</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Copper</td>
<td>0.73</td>
<td>18.50</td>
<td>1.74</td>
<td>1,211</td>
<td>2</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Iron</td>
<td>10.42</td>
<td>49.07</td>
<td>21.80</td>
<td>1,215</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Magnesium</td>
<td>594.0</td>
<td>1,940.0</td>
<td>1,190.1</td>
<td>1,217</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Manganese</td>
<td>1.69</td>
<td>14.30</td>
<td>6.19</td>
<td>1,216</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Phosphorus</td>
<td>1,470.0</td>
<td>5,330.0</td>
<td>3,271.3</td>
<td>1,309</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Potassium</td>
<td>1,810.0</td>
<td>6,030.0</td>
<td>3,848.1</td>
<td>1,217</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Selenium</td>
<td>0.05</td>
<td>0.75</td>
<td>0.21</td>
<td>184</td>
<td>98</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Sodium</td>
<td>0.17</td>
<td>731.54</td>
<td>32.36</td>
<td>1,152</td>
<td>935</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Sulfur</td>
<td>506.1</td>
<td>1,370.0</td>
<td>847.8</td>
<td>53</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Zinc</td>
<td>6.5</td>
<td>37.2</td>
<td>21.6</td>
<td>1,217</td>
<td>0</td>
<td>mg/kg DW</td>
</tr>
</tbody>
</table>

Copies easier into Excel
Report Options (Screen 4)

Select Report Format

In this section, you can choose the options that affect how the results of your search will be presented in the final report.

Start by choosing one of the predefined Report Types.

You must then choose an output format.

To print your report, use the pull-down menu labeled Output Format to save a PDF to your desktop, and then print the PDF from your desktop.

And lastly, you can optionally provide a Title and Description to display at the top of your report and choose whether to display the Query Criteria and Report Options on your final report.

You may go back and forth between this page and the final report to refine your output.
Data: *Detailed Report (Screen 5)*

### Tabular Report

#### Report Output

**Output Format:** HTML

#### Mineral Levels in Maize

- **search across all countries and years**
- **Query Criteria:**
  - Crop Type is Corn - Field - Maize - Zea mays
  - Tissue Type is Grain

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>&lt;LOQ (7.52)</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>&lt;LOQ (7.72)</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>&lt;LOQ (7.74)</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>&lt;LOQ (7.77)</td>
<td>mg/kg DW</td>
</tr>
<tr>
<td>Minerals</td>
<td>Cadmium</td>
<td>&lt;LOQ (7.77)</td>
<td>mg/kg DW</td>
</tr>
</tbody>
</table>

[< Revise Report Options] [New Query]
Overview of the General Trial Process

Idea

Question

Objectives

Planning & Design

Experiment
(one or more field trials)

Analysis

Interpretation

Conclusion

ACTION
Why do we conduct experiments?

The objective is to determine if there are real differences among responses to treatments, and . . .

to estimate the magnitude of those differences if they exist and . . .

to understand if the outcome is repeatable.
FACT:
As agricultural researchers, we work in some of the most variable experimental conditions in science.
Statistical inference of differences among treatments involves assigning some measure of probability to the inference.

How likely is it that an observed difference was a random occurrence?

$P = 0.05$ If the probability of something occurring at random is below 5%, then we say it is significant

Note: setting the probability at $P = 0.05$ means that we are willing to accept that 1 in 20 comparisons will result in concluding that a difference occurred, when it actually occurred by chance (a “false positive”).
Experimental Error

- Experimental error is the variation that exists among observations on experimental units (plots) \textit{treated alike}.
- Account for and remove \textit{known} variation when possible.
- Take steps to allow quantification of \textit{unknown} variation.

\textbf{If} \quad \text{Background Noise} \quad \textgreater \quad \text{Treatment Difference} \\
\textbf{Then} \quad \triangleright \quad \text{fail to detect a true difference}
Field Trial Design for Comparative Assessment Studies

● Entries / Treatments:

- **Control** - typically a nontransgenic, near-isogenic genotype, but depends upon nature of trait and availability of the genotype

- **GM event of interest**

- **Reference varieties** normally included –
  - commercial varieties, other germplasm sources
  - currently these are usually non-GM, but this may change
  - normally at least three; helps to assess variability in the crop
Field Trial Design for Comparative Assessment Studies

- **Number of locations:**
  - Need to be able to represent environmental growing conditions normal for that crop
  - Three locations usually a minimum
  - One year sufficient if environmental conditions can be represented

- **Randomized Complete Block Design (RCBD) is typical**
  - Multiple replications at each field trial site (4 is usually a minimum) and reps arranged into “blocks”
  - Each entry appears once and only once in each block; assigned randomly to each block
Statistical Model:

\[ Y_i = U + E_i \]
Statistical Model:

\[ Y_i = U + E_i \]

Observations:

\[
\begin{align*}
7 & = 9.2 + E_i \\
7 & = 9.2 + E_i \\
9 & = 9.2 + E_i \\
10 & = 9.2 + E_i \\
6 & = 9.2 + E_i \\
14 & = 9.2 + E_i
\end{align*}
\]
Statistical Model:

\[ Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk} \]
Statistical Model:

\[ Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk} \]

Identified sources of variability

Error
Experimental Error

A good experiment is one that both facilitates valid identification of experimental error and reduces experimental error.

Account for Experimental Error
- appropriate design & analysis
- adequate replication
- randomization
- proper blocking

Reduce Experimental Error
- uniform exp. units
- size/shape of exp. units
- unbiased sampling
- precise measurements
Orientation of blocks within the field design:

- **Blocked *against* a gradient**
  - The blocks (reps) cannot account for the differences within the field
  - Could lead to false differences among treatments

- **Sources of gradients:**
  - slope
  - soil factors (O.M., parent material, water movement)
  - fertilizer dispersal
  - movement of equipment
  - crop pests
  - unknowns….
Orientation of blocks within the field design:

- **Blocked with a gradient**
  - allows statistical model to account for differences in the gradient
  - Error term becomes smaller; more likely detection of true differences

- **Problems:**
  - More than one gradient possible (probable)
  - Gradient(s) often unknown
Interpretation of Data from Compositional Analysis Studies

Statistical differences should be investigated to determine relevance to nutrition and health
- Place differences in context of natural variability

- First data scenario – example of how to interpret results from a comparative analysis study

- Three additional data scenarios –
  - Work in groups of 2 or 3
  - Discuss results and what types of things to consider in data interpretation
  - Formulate a conclusion – has equivalence between the novel GM crop and the conventional crop been demonstrated?
## Composition of event NK603 maize (glyphosate-tolerant) at two replicated sites in the EU

<table>
<thead>
<tr>
<th>Component (percent dry weight)</th>
<th>NK603 mean (range)</th>
<th>Control mean (range)</th>
<th>Comm. Hybrids (range)</th>
<th>Literature (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.07 (10.23-13.92)</td>
<td>11.34 (10.13-13.05)</td>
<td>10.23-13.05 (7.77-12.99)</td>
<td>6.0-12.0 (9.7-16.1)</td>
</tr>
<tr>
<td>Total Fat</td>
<td>4.16 (3.87-4.48)</td>
<td>3.60 (3.24-3.84)</td>
<td>3.87-4.48 (2.57-4.95)</td>
<td>3.1-5.7 (2.9-6.1)</td>
</tr>
<tr>
<td>Ash</td>
<td>1.38 (1.23-1.65)</td>
<td>1.34 (1.25-1.50)</td>
<td>1.23-1.50 (1.02-1.94)</td>
<td>1.1-3.9 (1.1-3.9)</td>
</tr>
<tr>
<td>ADF</td>
<td>3.21 (2.63-3.87)</td>
<td>3.03 (2.30-3.68)</td>
<td>2.63-3.87 (2.46-6.33)</td>
<td>3.3-4.3 (3.3-4.3)</td>
</tr>
<tr>
<td>NDF</td>
<td>10.08 (8.50-12.00)</td>
<td>10.57 (9.35-11.63)</td>
<td>8.50-12.00 (8.45-14.75)</td>
<td>8.3-11.9 (8.3-11.9)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>82.39 (80.49-84.57)</td>
<td>83.73 (81.93-84.92)</td>
<td>80.49-84.57 (82.18-88.14)</td>
<td>not reported</td>
</tr>
</tbody>
</table>

Levels of total fat detected in NK603 maize compared to control


- NK603: 4.16%
- Control: 3.6%

Ranges:
- Literature Range (Watson): [4.16, 5.0]
- Literature Range (Jugenheimer): [5.5, 6.5]
- ILSI database: [6.0, 7.0]

Notes:
## Composition of event NK603 maize (glyphosate-tolerant) at two replicated sites in the EU

<table>
<thead>
<tr>
<th>Component (mg/kg dry weight)</th>
<th>NK603 mean (range)</th>
<th>Control mean (range)</th>
<th>Comm. Hybrids (range)</th>
<th>Literature (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.0053 (0.0050-0.0058)</td>
<td>0.0053 (0.0050-0.0058)</td>
<td>0.0039-0.0076</td>
<td>0.01-0.1</td>
</tr>
<tr>
<td>Copper</td>
<td>1.89 (1.77-1.99)</td>
<td>1.83 (1.69-1.97)</td>
<td>1.16-2.78</td>
<td>0.9-10</td>
</tr>
<tr>
<td>Iron</td>
<td>22.73 (17.43-26.91)</td>
<td>21.81 (18.52-25.87)</td>
<td>15.42-29.34</td>
<td>1-100</td>
</tr>
<tr>
<td>Manganese</td>
<td>6.73 (5.18-7.90)</td>
<td>6.42 (5.63-7.32)</td>
<td>3.86-10.47</td>
<td>0.7-54</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.36 (0.34-0.38)</td>
<td>0.38 (0.36-0.39)</td>
<td>0.32-0.45</td>
<td>0.32-0.72</td>
</tr>
<tr>
<td>Zinc</td>
<td>23.78 (15.95-31.45)</td>
<td>23.21 (17.87-29.88)</td>
<td>13.51-27.98</td>
<td>12-30</td>
</tr>
</tbody>
</table>

### Composition of event MON 88017 maize (glyphosate-tolerant) at two replicated sites in Argentina

<table>
<thead>
<tr>
<th>Component (% of total fatty acids)</th>
<th>MON 88017 mean (range)</th>
<th>Control mean (range)</th>
<th>Comm. Hybrids (range)</th>
<th>Literature (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 palmitic</td>
<td>11.52 (11.31-11.79)</td>
<td>11.71 (11.35-12.58)</td>
<td>(8.29-12.81)</td>
<td>(8.51-17.46)</td>
</tr>
<tr>
<td>18:0 stearic</td>
<td>2.05 (2.00-2.11)</td>
<td>2.07 (1.95-2.20)</td>
<td>(1.35-2.49)</td>
<td>(1.02-2.76)</td>
</tr>
<tr>
<td>18:1 oleic</td>
<td>26.61 (25.72-27.74)</td>
<td>32.12 (30.50-33.97)</td>
<td>(19.73-40.72)</td>
<td>(18.6-40.1)</td>
</tr>
<tr>
<td>18:2 linoleic</td>
<td>57.69 (56.22-58.80)</td>
<td>51.97 (49.67-53.98)</td>
<td>(45.41-65.50)</td>
<td>(43.1-65.6)</td>
</tr>
<tr>
<td>18:3 linolenic</td>
<td>1.12 (1.08-1.16)</td>
<td>1.08 (0.92-1.14)</td>
<td>(0.73-1.30)</td>
<td>(0.70-1.92)</td>
</tr>
<tr>
<td>20:0 arachidic</td>
<td>0.43 (0.41-0.44)</td>
<td>0.45 (0.42-0.49)</td>
<td>(0.30-0.53)</td>
<td>(0.279-0.720)</td>
</tr>
</tbody>
</table>

From: McCann et al., J. Agric. Food Chem. 2007, 55, 4034-4042
### Mesotrione mode of action

**Plastoquinone/Tocopherol Synthesis**

- **Tyrosine** → **4-Hydroxyphenylpyruvate**
- **Homogentisate**
- **2-Demethylphytol-plastoquinone**
- **2-Demethylplastoquinone**
- **Vitamin E**
- **Plastoquinone**

**Mesotrione**

**p-HPPD**

#### Literature:
Overexpression of *hppd* may affect vitamin E levels

**Event SYHT0H2** - includes *avhppd-03* gene from oat (less binding affinity for mesotrione)

#### Vitamin E isoforms and relative activity:

<table>
<thead>
<tr>
<th>Vitamin E isoforms</th>
<th>Relative Vitamin E activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>100%</td>
</tr>
<tr>
<td>β-tocopherol</td>
<td>50%</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>10%</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>3%</td>
</tr>
</tbody>
</table>

Further investigation of these levels
Composition of event SYHT0H2 soybean (mesotrione-tolerant) at eight replicated sites in the USA

<table>
<thead>
<tr>
<th>(µg/g seed dry weight)</th>
<th>SYHT0H2 mean (range)</th>
<th>Control mean (range)</th>
<th>non-GM Reference Varieties mean (range)</th>
<th>Codex Alimentarius (standard ranges in soybean oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>22.8 (9.96-62.8)</td>
<td>25.8 (9.34-60.5)</td>
<td>29.5 (11.5-77.1)</td>
<td>(9-352)</td>
</tr>
<tr>
<td>β-tocopherol</td>
<td>&lt; LOQ</td>
<td>&lt; LOQ</td>
<td>-</td>
<td>(&lt; LOQ-7.79) (undetectable-36)</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>226 (183-268)</td>
<td>201 (154-244)</td>
<td>175 (127-236)</td>
<td>(89-2307)</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>78.9 (51.8-107)</td>
<td>61.1 (31.2-84.5)</td>
<td>68.7 (32.0-112)</td>
<td>(154-932)</td>
</tr>
</tbody>
</table>