



Crop Composition as Part of the GM Crop Safety Assessment

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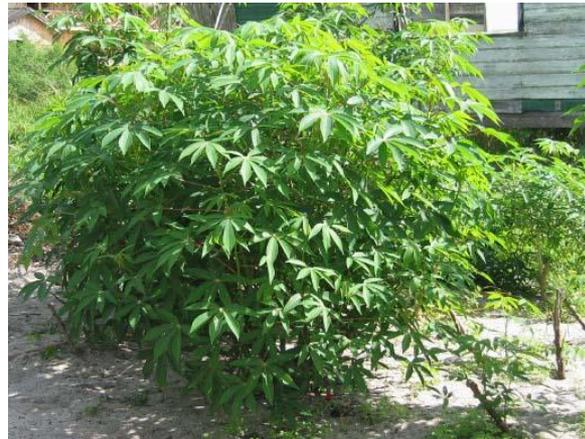
World-wide adaptation of biotech crops

- 100-fold increase in area planted to GM crops since 1996 (1.7 million ha in 1996 vs. 175 million ha in 2013)
- Biotech (GM) crops fastest adopted crop technology in recent history (James, 2012)



Many countries require comprehensive safety assessment of the GM crop

- General guidelines for the safety assessment were 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



Comparative Assessment

Safety assessment

- Mode of action
- Agronomic evaluation
- Compositional analysis
- Protein expression studies
- Allergenicity assessments
- Molecular characterization
- Specific toxicity studies
- Environmental risk assessment

Comparative assessment

Comparative assessments contribute to the overall safety assessment.



Why compositional analysis?

- *Hypothesis:* the insertion of a new GM trait into the genome may result in a gain, alteration of expression, or loss of a trait.
 - This may have the potential to influence the composition of the GM crop relative to its non-GM parent line.
- Therefore, compositional studies measure the levels of key nutritional components within the GM crop and compare those levels to the non-GM crop.



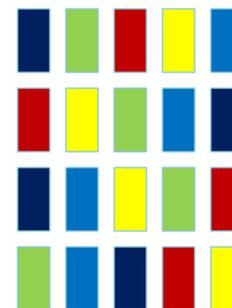
Goal of Comparative Assessment: Composition

- 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 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



General Composition Study Design

- Typical entries / treatments:
 - GM crop of interest
 - Non-GM comparator (usually genetically similar to GM crop)
 - Commercial varieties (reference varieties); normally 3 or more
- Randomized complete block design (RCBD)
- 3 to 4 replicates (blocks) per field trial site
- Multiple field trial sites
- Samples collected as appropriate for the crop (on a per plot basis, not pooled) – **Analysis of Samples**



What Components are Measured?

- 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



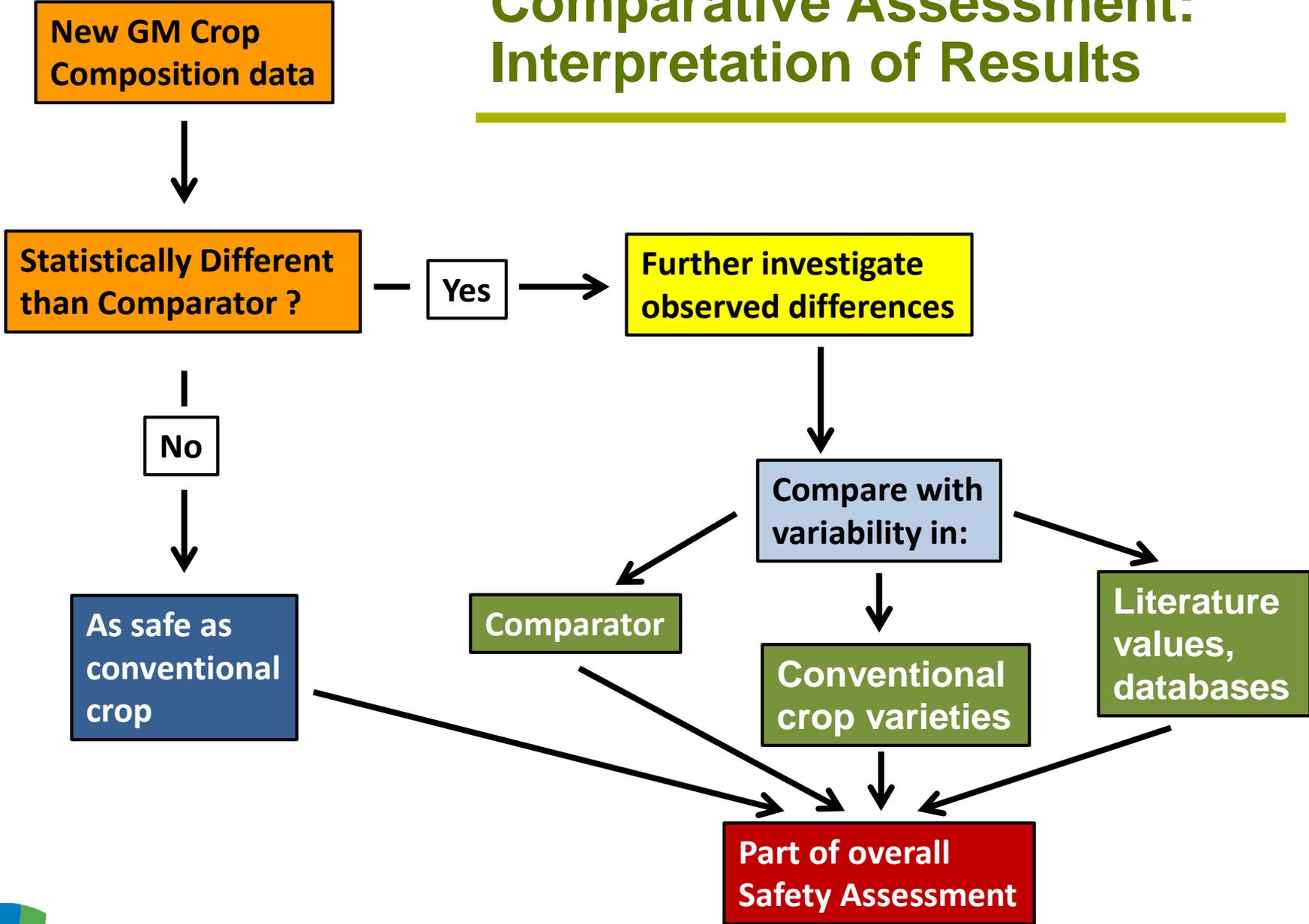
OECD Recommended Components for Compositional Analysis of Maize and Cassava

Maize		Cassava	
Forage	Grain	Leaf	Root
Crude protein	Crude protein	Crude protein	Crude protein
Fat	Fat	Fat	Fat
Ash	Ash	Ash	Ash
Carbohydrates	Carbohydrates	Carbohydrates	Carbohydrates
ADF, NDF, TDF	ADF, NDF, TDF	ADF, NDF, TDF	ADF, NDF, TDF
Minerals (Ca, P)	Minerals	Minerals	Minerals
	Vitamins	Cyanogenic glycosides ^a	Vitamins
	Amino acids	Hydrocyanic acid	Amino acids
	Fatty acids	Phytic acid	Fatty acids
	Furfural	Tannins	Cyanogenic glycosides ^a
	Ferulic acid		Hydrocyanic acid
	p-coumaric acid		
	Phytic acid		
	Raffinose		

^a Cyanogenic glycosides include linamarin and lotaustralin.



Comparative Assessment: Interpretation of Results



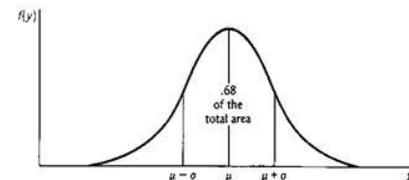
Assessing Biological Relevance

“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.”

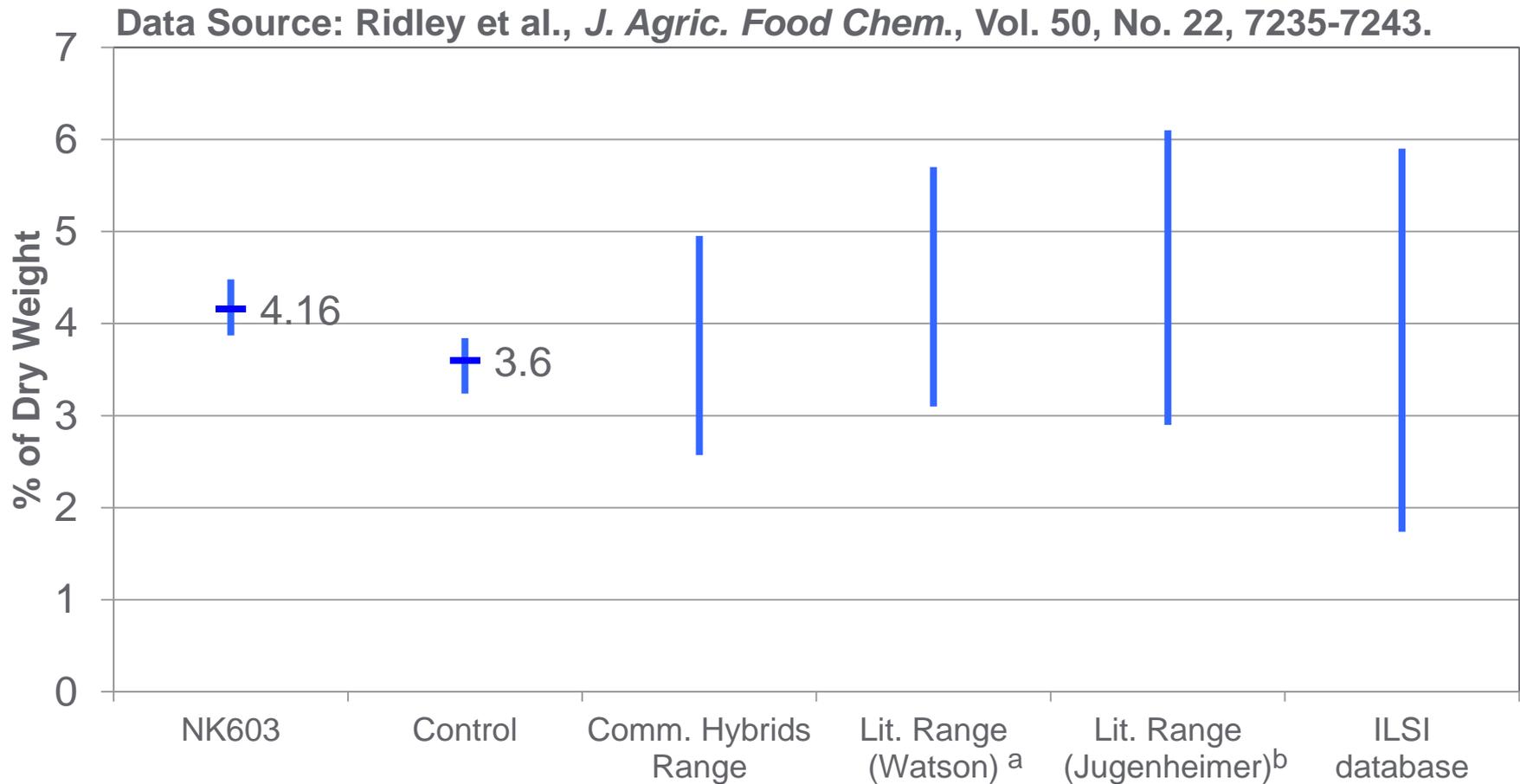
Codex Alimentarius. 2003. Guideline For The Conduct Of Food Safety Assessment Of Foods Derived From Recombinant-DNA Plants. CAC/GL 45-2003.

Statistical significance \neq Biological relevance

There may be statistically significant differences between two non-GM varieties of a crop, but they are considered to be substantially equivalent to one another.



Levels of total fat detected in NK603 maize compared to control



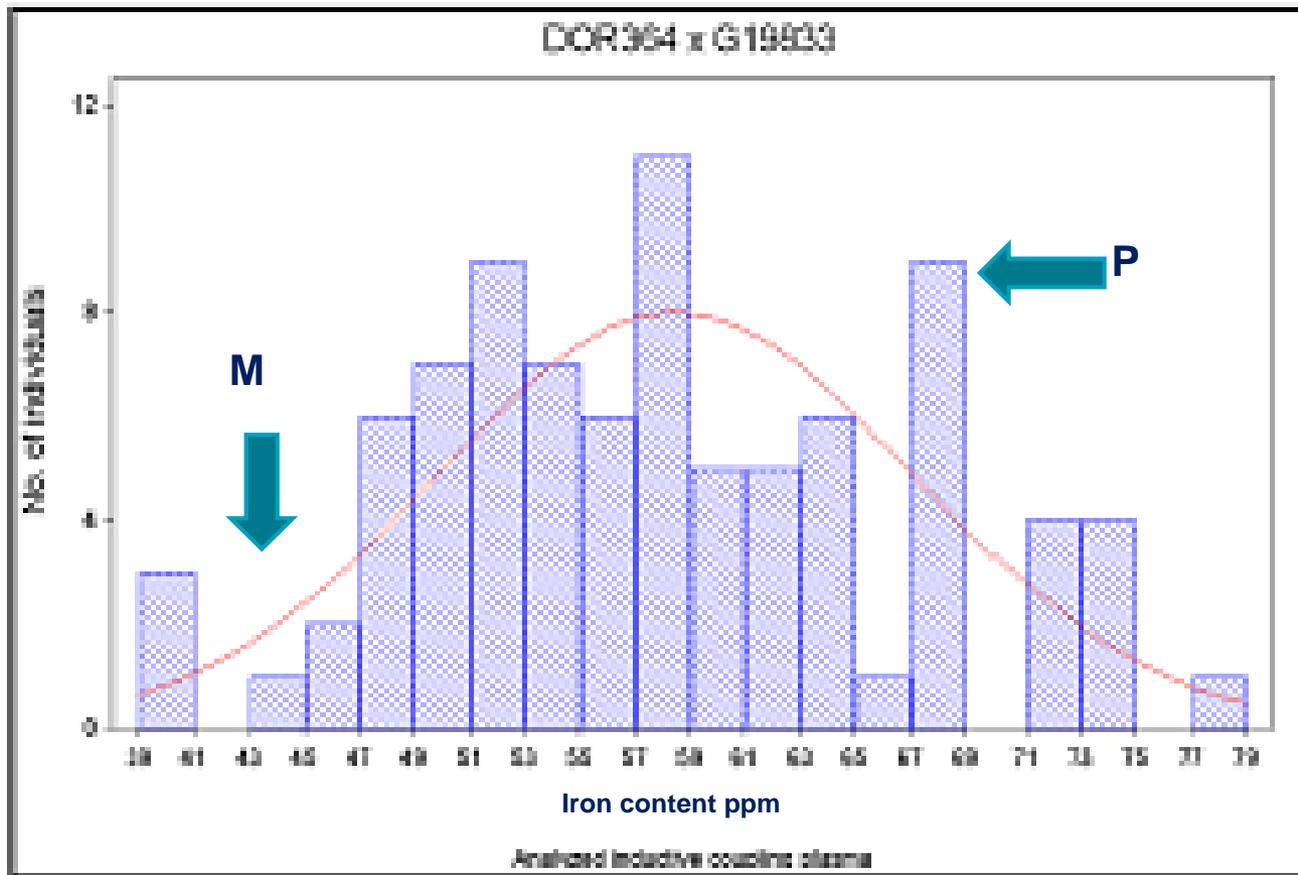
^a Watson, Stanley A. Structure and composition. In *Corn: Chemistry and Technology*; Watson, S. A., Ransted, P. E., Eds.; American Association of Cereal Chemists: Minneapolis, MN, 1987; pp 53-82.

^b Jugenheimer, R. W. In *Corn: Improvement, Seed Production, and Uses*; Wiley: New York, 1976; p 227.



Sources of Variation in Crop Composition

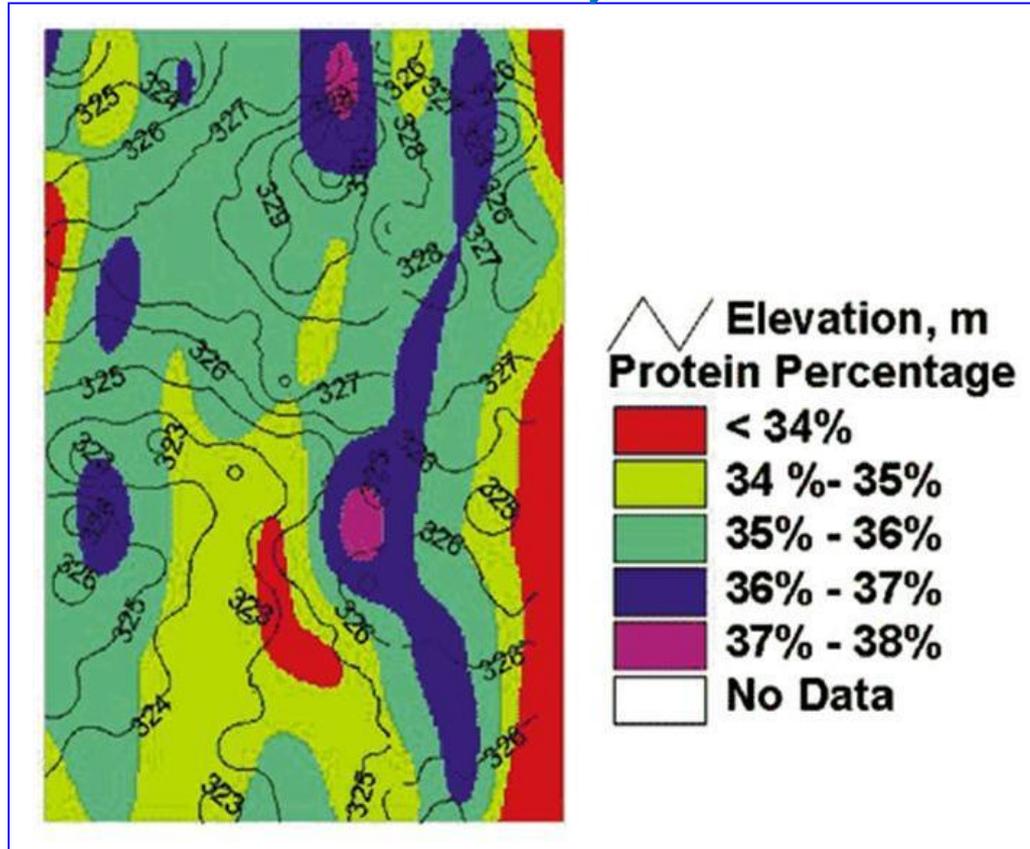
Fe content in common bean



Genotype

Sources of Variation in Crop Composition

Protein content in soy across a field



Environment

Sources of Variation in Crop Composition

Planting Methods May Affect Component Levels

“The seeding date had a greater impact [than seeding rate and row spacing]; mid- to late-May seeding across four environments resulted in 45% greater α -tocopherol concentrations than seeding at later dates.”

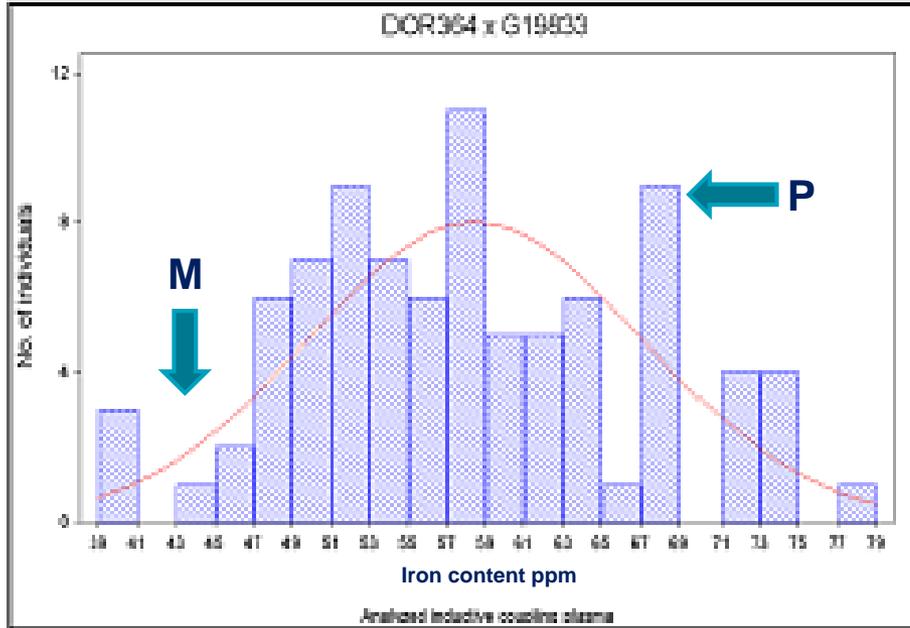
Seguin, P. *et al.*, J. Agric. Food Chem.,
Vol. 58, No. 9, 2010

Environment

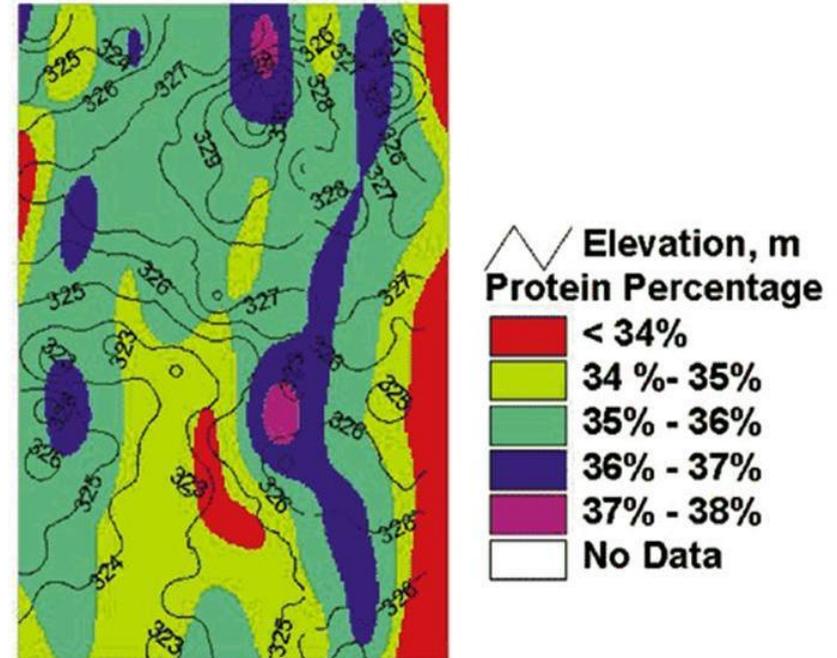


Sources of Variation in Crop Composition

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



Maize Landraces

From: Flint-Garcia, ILSI CCW, 2012



Global Commodities



**Compare to variability
in the crop commodity**



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Interpretation of data should be with respect to the safety assessment

- Place suitable emphasis on comparison of GM with comparator
 - Differences between GM crop and its comparator may be due to some unforeseen bias (GM and comparator not as genetically similar as assumed, background germplasm, field conditions, bias in sampling, incorrect plot layout...) or by chance.
- Ask questions that are important in determining food/feed safety and quality, and avoid those driven by scientific interest
 - Compositional differences may not be relevant to the issue of safety (same differences may be present among conventional varieties)



Evaluation of Traits Stacked Through Conventional Breeding

Parent **Parent**
Herbicide-tolerant event Insect-resistant event



F1 offspring
HT x IR



Both events have been approved



Assess:
Is there a potential for interaction?



Yes:
Form hypothesis and investigate



No:
No need to reassess the stack



Summary

- Comparative assessment contributes to the overall safety assessment of the novel GM crop
- Compositional analysis is a nutritional assessment: any statistically significant differences between the GM and the comparator are placed in context of the natural variability in the crop having a history of safe use
- Variation in component levels is inherent in all crops
 - Genetic background, environment, interactions
 - *Value ranges for reference varieties* grown within a study, *literature values*, and *databases* are very important in helping to place observed differences in the context of variability within the crop



Summary

- Statistically Significant Differences do not mean that the crop is unsafe
- Evaluation of stacked traits created through conventional breeding
 - If events in the stack have already been assessed, then there is no need to carry out an additional safety assessment unless some interaction of event products is likely
 - To date, no safety issues have been identified in stacked traits



Thank You!

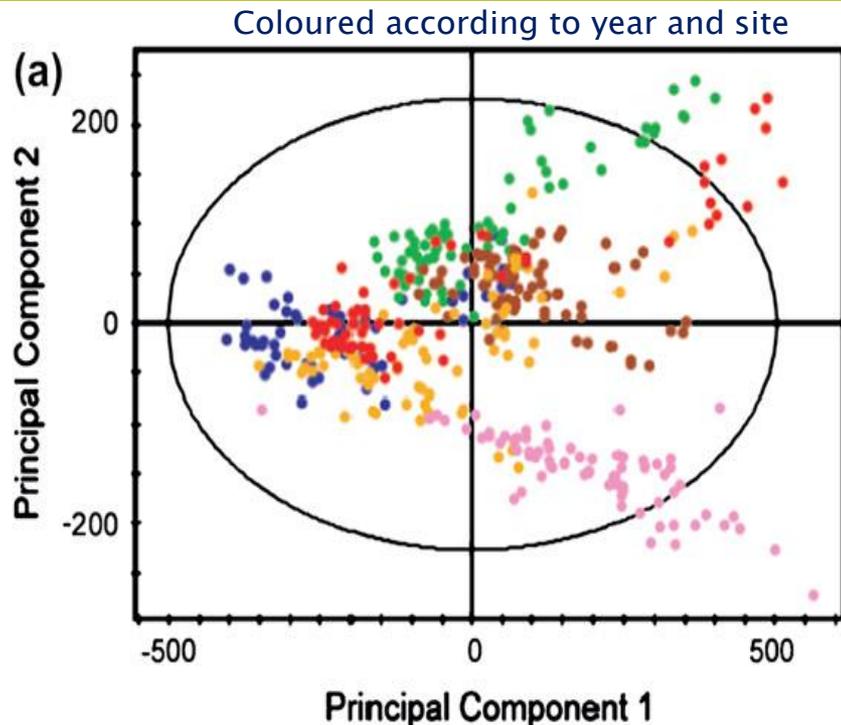


Compositional Analysis Moving Forward

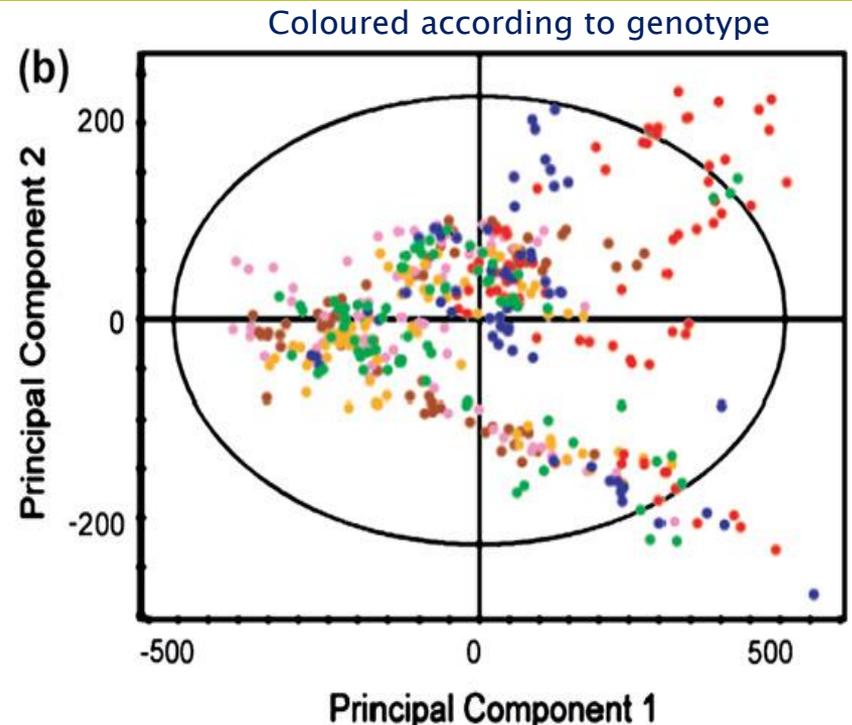
- With 20 years experience working with safety of GM crops, perhaps the compositional analysis approach could take a different focus that reflects our experience:
 - Do we need all data in all cases?
 - Assess by crop (experience has shown GM maize to be safe; “newer” crops may need more thorough evaluation)
 - Assess by trait (experience has shown current mechanisms of insect resistance traits to be safe; glyphosate-related herbicide traits safe)
 - Modify OECD-suggested list of components depending on trait and experience with the crop (Hypothesis-driven decisions about what to include; what makes sense for mode-of-action)



Environment is larger contributor to compositional variability than genotype (transgenic and control wheat lines)



Rothamsted 2001 pink
2000 brown
1998 red
Long Ashton 2001 orange
2000 green
1998 blue



Control lines
L88- 6 blue
L88-31 green.
Transgenic lines
B73-6-1 derived from L88-6 red
B72-8-11a derived from L88-31 orange
B102-1- 1 derived from L88-31 brown
B102-1-2 derived from L88-31 pink

