



# Sources of variability in measuring aflatoxin and the role of sampling

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Texas Feed and Fertilizer Control Service • Agriculture Analytical Service



# Sampling

Laboratory

Reagents

Analyst

## Sources of Variability

Sample reduction

Testing platform

Sub-sample measurement

Scale

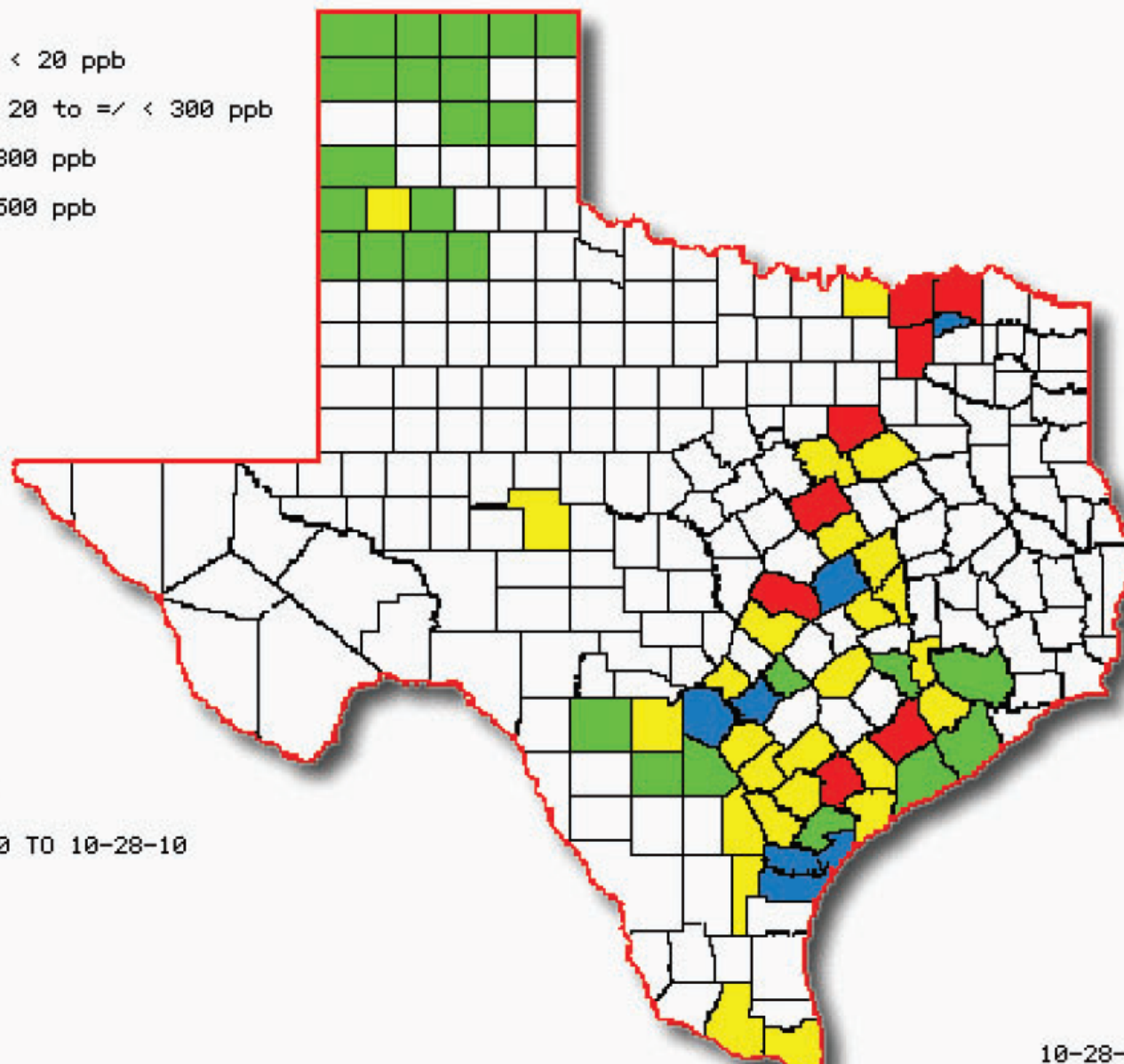
Detection

Sample matrix



### Highest Level of Aflatoxin in Corn Sampled & Tested by OTSC - 2010 Crop Year By County

- - = < 20 ppb
- - > 20 to =/ < 300 ppb
- - >300 ppb
- - >500 ppb



LEGEND:

07-22-10 TO 10-28-10

# One-Sample Strategy Program Components

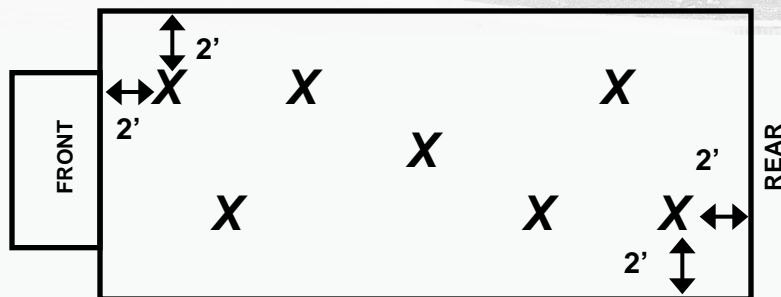


# Criteria: Sampling

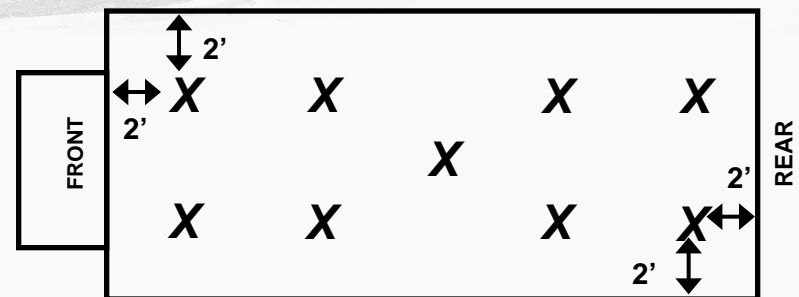
- Minimum 5-pound sample collected from each incoming truck or trailer
- USDA representative sampling patterns
- 6' spiral hand probe



**PATTERN 1:** 7 probes for trucks or trailers loaded with grain more than 4 feet deep



**PATTERN 2:** 9 probes for trucks or trailers loaded with grain less than 4 feet deep



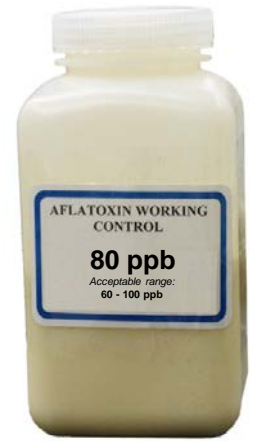
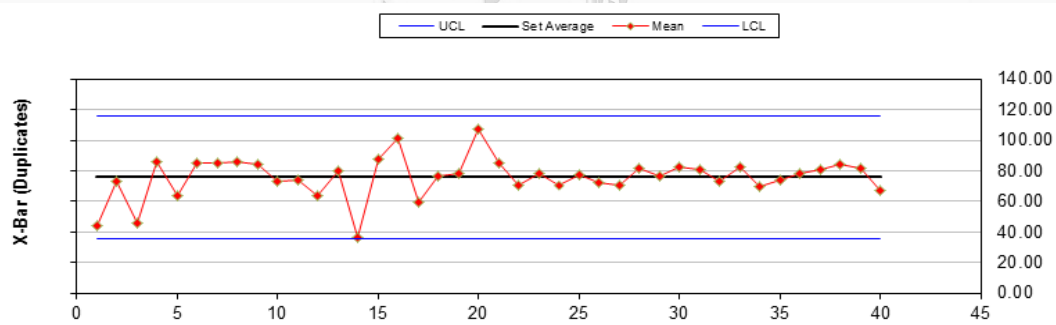
# Criteria: Grinding

- ❑ Grind the entire sample
- ❑ Collect at least 500 grams of the ground sample
- ❑ 70% of the particles pass through a 20 mesh sieve after grinding

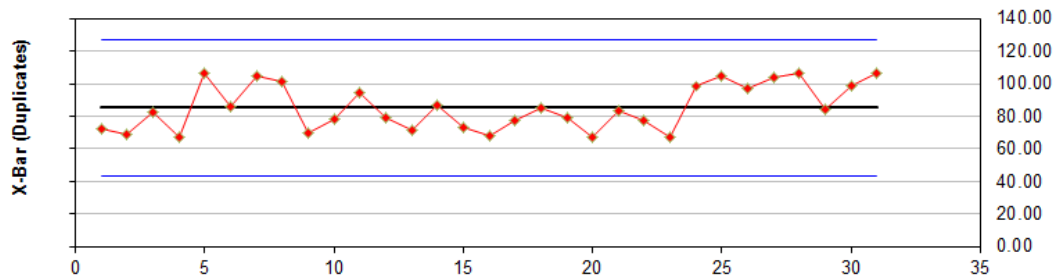


# Control Chart

Company  
A



Company  
B



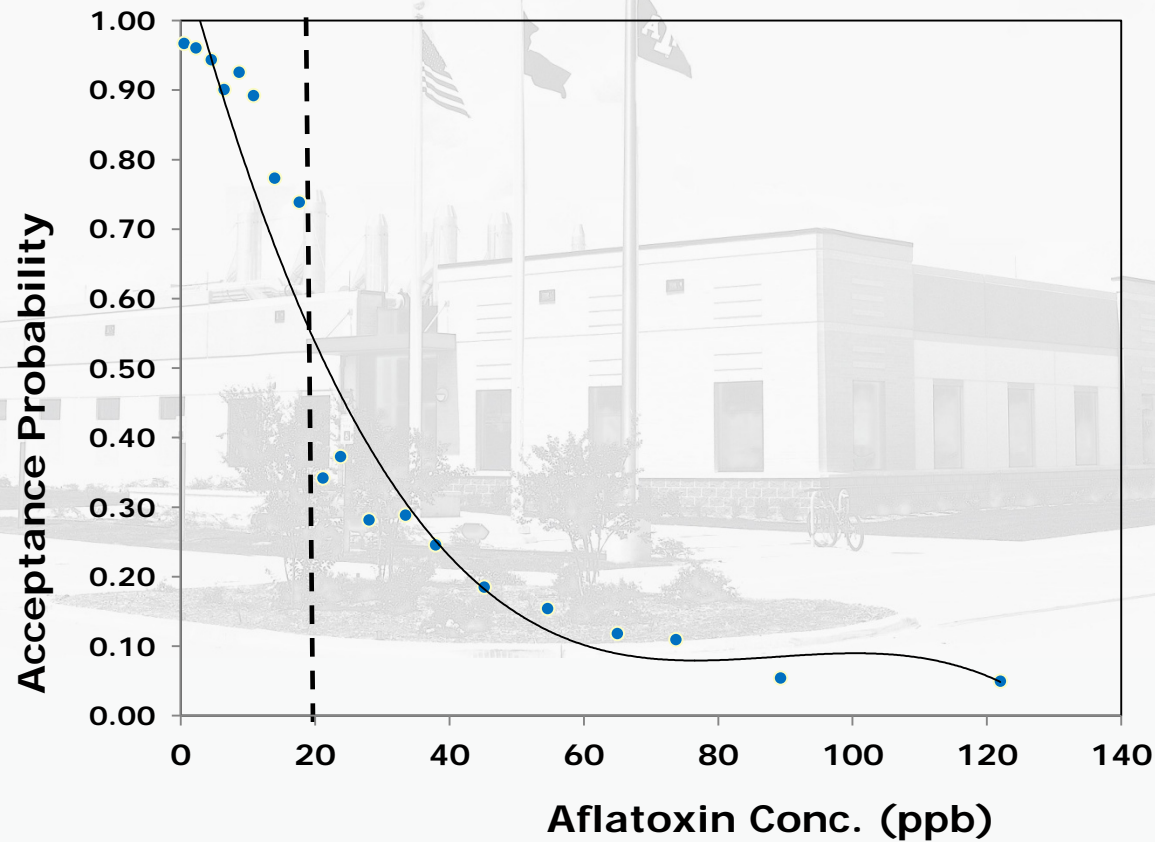


# OTSC Monitoring

- ❑ Employee performance
- ❑ Equipment performance
  - Grinder check
  - Lab scale check
- ❑ Control standard record
- ❑ Retained sample analysis in an ISO 17025 accredited lab



# Performance curve for 2013-2015



A faded background image of a modern, single-story building with large windows and a flat roof. In front of the building, there are three tall flagpoles. The leftmost pole has the American flag, the middle pole has a dark flag, and the rightmost pole has a flag with a white 'A' on a dark background. There are also some small trees and a wheelchair in the foreground.

Inference about the population

# **SAMPLING**

## Variance Structure of Aflatoxin Contaminated Maize in Commercial Grain Elevators and Transporters

Variance Source	Percent of Total Variance
Facility	1.9
Bin	65.8
Truck	9.1
Sampling and Testing Error	23.2



## Variance Structure of Aflatoxin Contaminated Maize in Commercial Maize Mills in Kenya

Variance Source	Percent of Total Variance
Mill	0
Truck	7.8
Bag	33.3
Within bag	50.1
Analytical	3.4
Error	5.4





Retaining the representative property of the sample

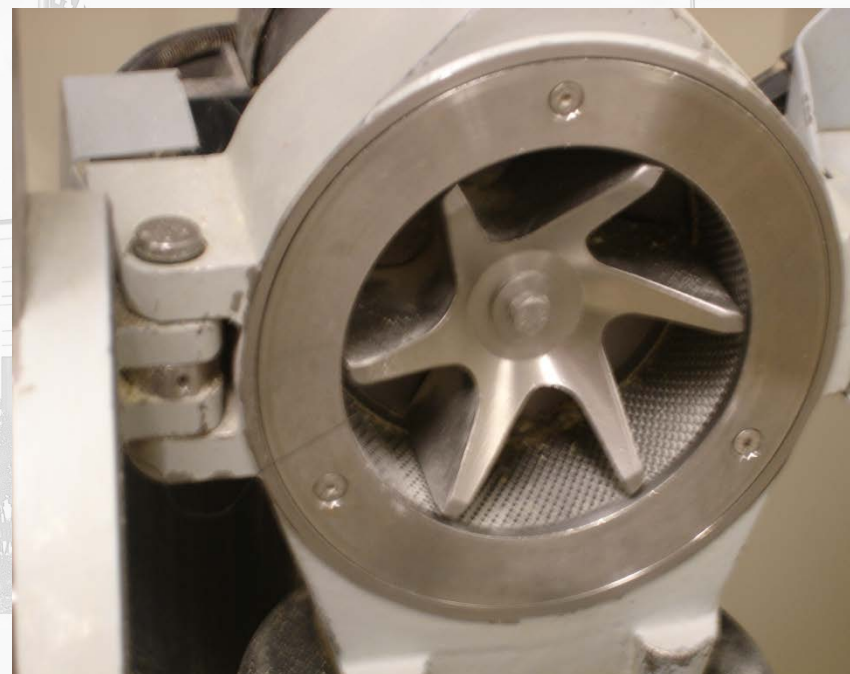
# GRINDING



# Sample Grinding



# Sample Grinding











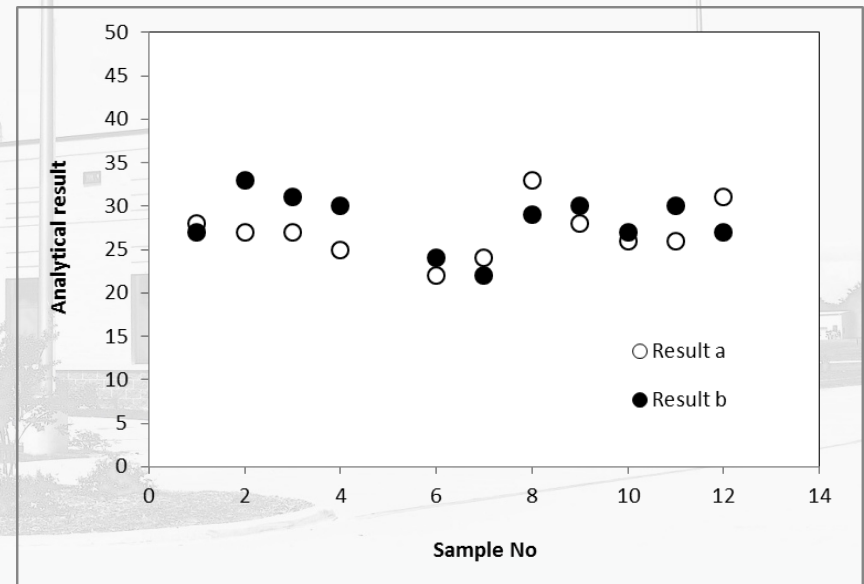
Developing uniform working controls

# REFERENCE MATERIAL



# Recommendation 9: Sufficient Homogeneity

In testing for sufficient homogeneity, duplicate results from a single distribution unit should be deleted before the analysis of variance if they are shown to be significantly different from each other by Cochran's test at the 99% level of confidence



## Sufficient stability

Changes in test material are inconsequential

Period in question is the interval between preparation of the material and the deadline for return of the results

5 samples will be analyzed after the proficiency test





Laboratory uncertainty

# UNCERTAINTY & VARIABILITY

# Uncertainty

## ISO 17025 5.4.6.2

- Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement...
- Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data

## Uncertainty Budget

- List all potential factors affecting variability in measurements –make table
- Determine the standard uncertainty for each factor including distribution
- Perform root sum squares for all factors to create the combined or standard uncertainty

$$S_I = \sqrt{S_a^2 + S_b^2 \dots S_x^2}$$

- Multiply by coverage factor: 2



# OTSC Uncertainty Measurement Estimation

Analyte	Procedure	Mean	Std. Dev.	CV	Uncertainty
Aflatoxin	HPLC	21.6	2.2	10.1	20.2
Aflatoxin	ELISA	24.6	3.7	15.1	30.3
Aflatoxin	LC/MS/MS	22.7	3.0	13.4	26.8
Aflatoxin	UHPLC	21.8	3.3	15.0	30.1
Aflatoxin	Fluoroquant	22.5	3.2	14.0	28.0
Fumonisin	LC/MS/MS	7.8	0.08	8.8	17.7



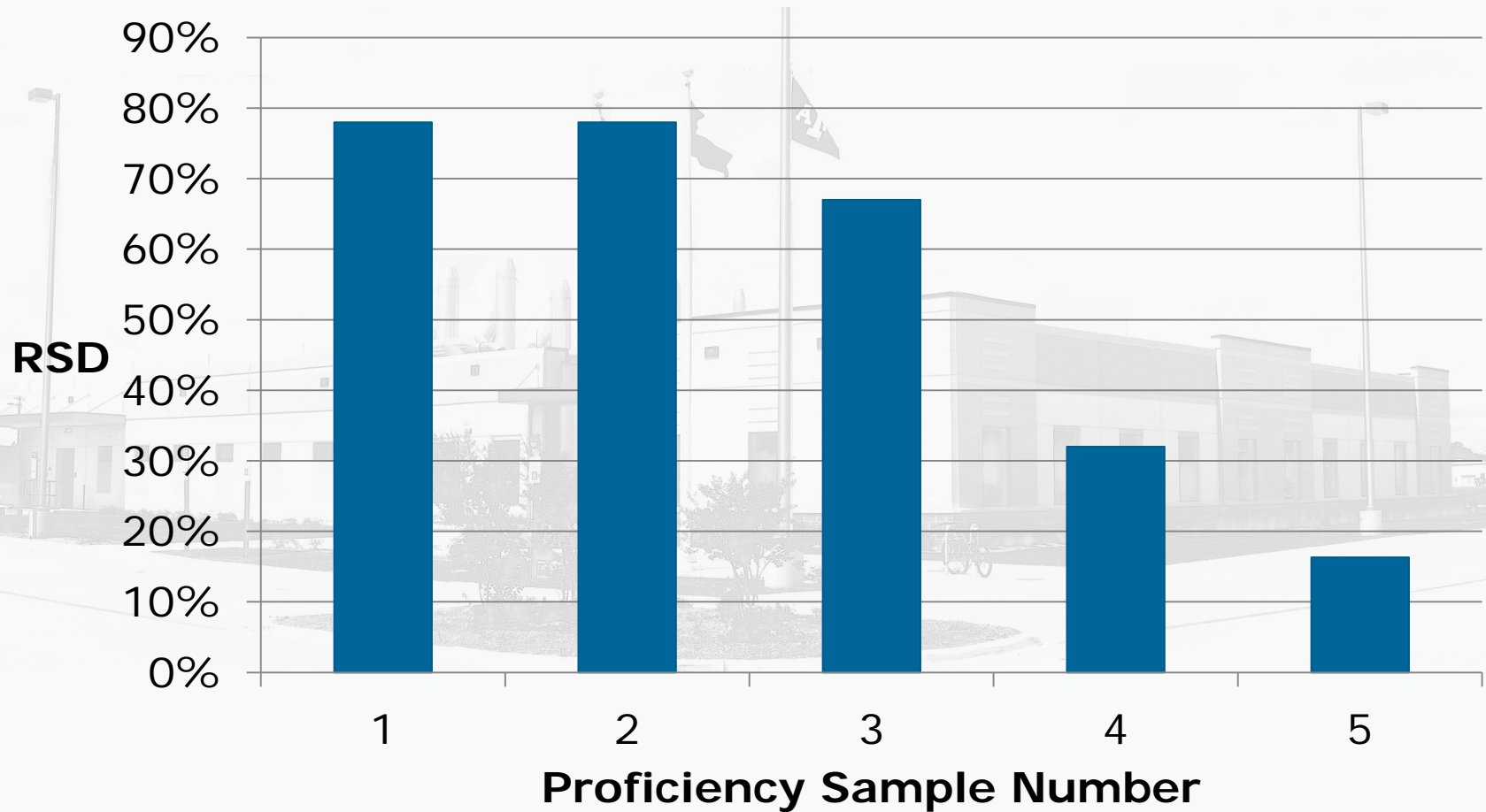
One of the Big Three

# PROFICIENCY TEST RESULTS

**APTECA Proficiency Testing Program**

**Corn Meal Sample #4**

# Proficiency Testing Performance






APTECA group qualification exercises

# LABORATORY ENVIRONMENT

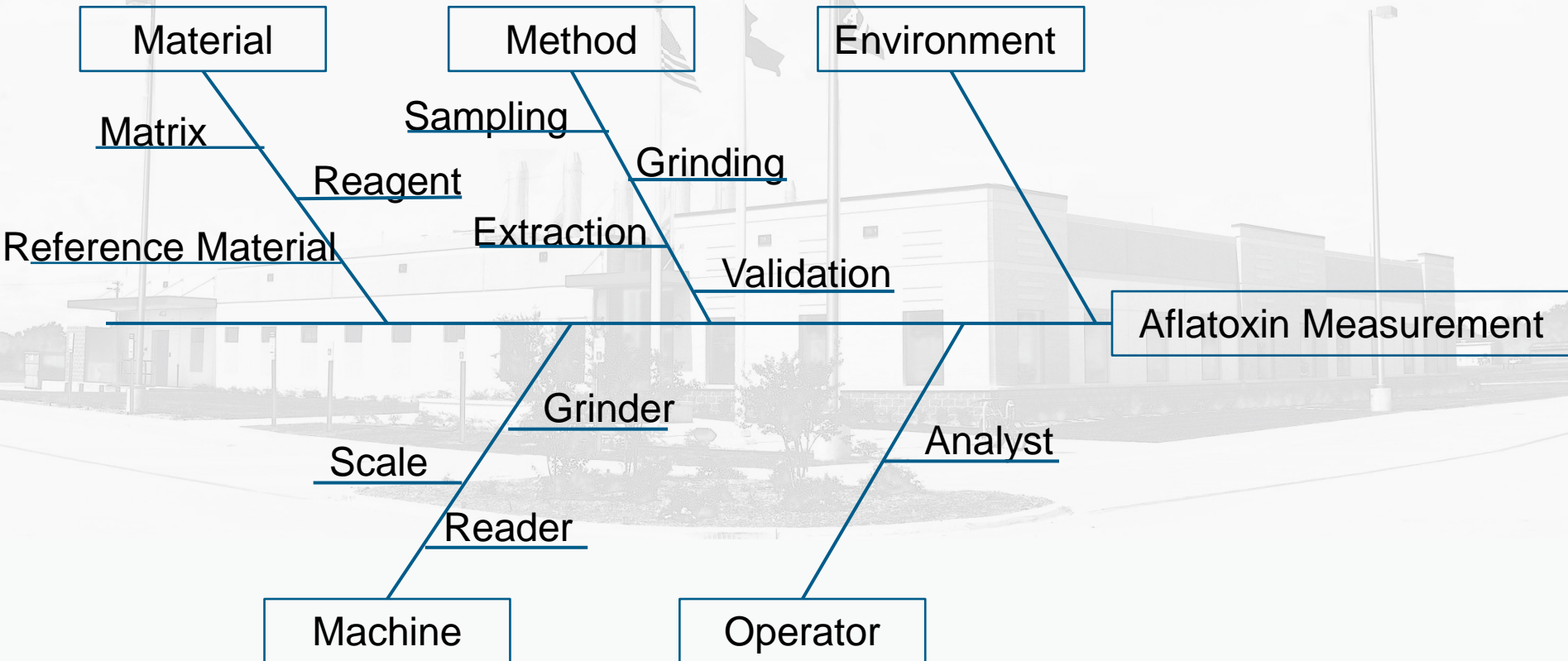


A faded background image of a modern, single-story building with a flat roof and large windows. In front of the building are three tall flagpoles. The leftmost pole has the American flag, the middle pole has a dark flag, and the rightmost pole has a white flag with a blue emblem. The building is surrounded by a paved area and some landscaping.

Sources of Variability

# SUMMARY

# Cause and Effect Diagram





# Uncertainty Budget for Total Variability

## Sources of Variability

- Sampling CV = 82%
- Test method CV = 46%
- Analyst CV = 32%

## Uncertainty Budget

$$S_I = \sqrt{S_a^2 + S_b^2 \dots S_x^2}$$

$$S_I = \sqrt{82_a^2 + 46_b^2 + 32_c^2}$$

$$S = 99\%$$

Worse Case

# Uncertainty Budget for Total Variability

## Sources of Variability

- Sampling CV = 23%
- Test method CV = 16%
- Analyst CV = 16%

## Uncertainty Budget

$$S_I = \sqrt{S_a^2 + S_b^2 \dots S_x^2}$$

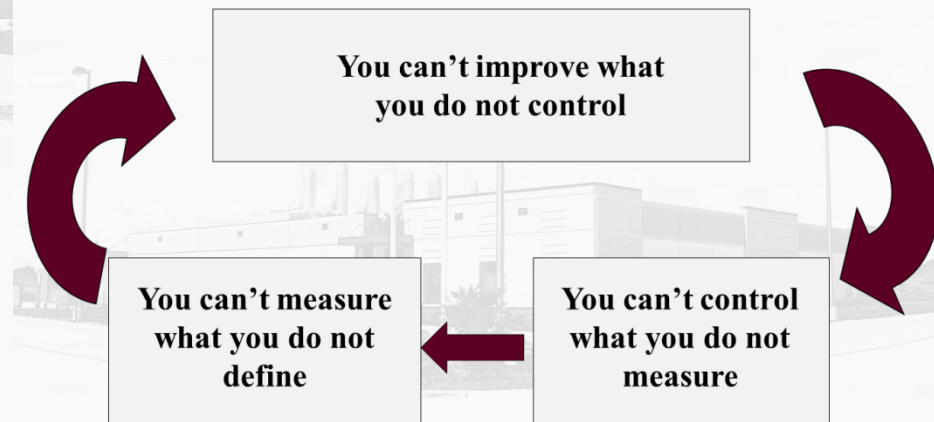
$$S_I = \sqrt{23_a^2 + 16_b^2 + 16_c^2}$$

$$S = 32\%$$

Best Case

## SOURCES OF VARIABILITY IN MEASURING AFLATOXIN AND THE ROLE OF SAMPLING

A continuous improvement approach to define, measure, and control aflatoxin helped reduce food safety risk.





# Acknowledgements

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