Sources of variability in measuring aflatoxin and the role of sampling

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Sources of Variability

Sampling

- Sample reduction
- Sub-sample measurement
- Sample matrix

Laboratory

- Analyst
- Reagents
- Testing platform
- Scale
- Detection
One-Sample Strategy Program Components

- Standardized methods
- Standardized training
- Verification of employee performance
- Documented program outcomes
- Monitoring & corrective actions
- Reduced market and food safety risk
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

OFFICE OF THE TEXAS STATE CHEMIST
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
Inference about the population

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

<table>
<thead>
<tr>
<th>Variance Source</th>
<th>Percent of Total Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility</td>
<td>1.9</td>
</tr>
<tr>
<td>Bin</td>
<td>65.8</td>
</tr>
<tr>
<td>Truck</td>
<td>9.1</td>
</tr>
<tr>
<td>Sampling and Testing Error</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Herrman et al. JRS 1(1):23-31
### Variance Structure of Aflatoxin Contaminated Maize in Commercial Maize Mills in Kenya

<table>
<thead>
<tr>
<th>Variance Source</th>
<th>Percent of Total Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill</td>
<td>0</td>
</tr>
<tr>
<td>Truck</td>
<td>7.8</td>
</tr>
<tr>
<td>Bag</td>
<td>33.3</td>
</tr>
<tr>
<td>Within bag</td>
<td>50.1</td>
</tr>
<tr>
<td>Analytical</td>
<td>3.4</td>
</tr>
<tr>
<td>Error</td>
<td>5.4</td>
</tr>
</tbody>
</table>
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
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
  \[ S_I = \sqrt{S_a^2 + S_b^2 \ldots S_x^2} \]
- Multiply by coverage factor: 2
## OTSC Uncertainty Measurement Estimation

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Procedure</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>CV</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>HPLC</td>
<td>21.6</td>
<td>2.2</td>
<td>10.1</td>
<td>20.2</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>ELISA</td>
<td>24.6</td>
<td>3.7</td>
<td>15.1</td>
<td>30.3</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>LC/MS/MS</td>
<td>22.7</td>
<td>3.0</td>
<td>13.4</td>
<td>26.8</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>UHPLC</td>
<td>21.8</td>
<td>3.3</td>
<td>15.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Fluoroquant</td>
<td>22.5</td>
<td>3.2</td>
<td>14.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Fumonisin</td>
<td>LC/MS/MS</td>
<td>7.8</td>
<td>0.08</td>
<td>8.8</td>
<td>17.7</td>
</tr>
</tbody>
</table>
One of the Big Three

PROFICIENCY TEST RESULTS
APTECA Proficiency Testing Program

Corn Meal Sample #4
Proficiency Testing Performance

<table>
<thead>
<tr>
<th>Proficiency Sample Number</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>70%</td>
</tr>
<tr>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td>4</td>
<td>50%</td>
</tr>
<tr>
<td>5</td>
<td>40%</td>
</tr>
</tbody>
</table>
SUMMARY

Sources of Variability
Cause and Effect Diagram

Material
  - Matrix
  - Reference Material

Method
  - Sampling
  - Reagent
  - Extraction

Environment
  - Grinding
  - Validation

Aflatoxin Measurement

Grinder
  - Scale
  - Reader

Machine
  - Operator

Analyst
Uncertainty Budget for Total Variability

Sources of Variability

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

Uncertainty Budget

\[ S_I = \sqrt{S_a^2 + S_b^2 + \ldots + S_x^2} \]

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

\[ S = 99\% \]
Uncertainty Budget for Total Variability

Sources of Variability

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

Uncertainty Budget

\[
S_I = \sqrt{S_a^2 + S_b^2 + \ldots + S_x^2}
\]

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

Best Case

\[S = 32\%\]
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

Office of the Texas State Chemist Personnel

Cindy McCormick, Carlton Peterson
K.M. Lee, Susie Dai, Anne Muiruri

Cereal Millers Association & members

Others

Vivian Hoffman
Jagger Harvey
Appoliniare Djikeng
Josephine Birungi
Harinder Makkar
Rosemary Bichara
Charles Manara