



# Variance structure of aflatoxin contaminated maize in commercial grain elevators and transporters

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## ABSTRACT

The application of risk analysis to manage chemical hazards in the grain industry by regulators presents significant challenges including development of sampling schemes and disposition plans in the presence of high levels of aflatoxin contamination. In this study, a firm comprised of seven grain elevators with 38 storage bins containing aflatoxin contaminated maize were studied to evaluate the risk management effectiveness of a sampling strategy negotiated in bankruptcy court. Samples from 551 incoming trucks and 301 outbound trucks of maize were analyzed for aflatoxin by Grain Inspection and Packers Stockyard Administration (GIPSA) Official Inspection Agencies (OIA). A comparison of the average aflatoxin measures for all incoming and outgoing trucks were 373 and 376 µg/kg, respectively. A comparison of 64 outbound trucks between the GIPSA OIA and the Office of the Texas State Chemist (OTSC) revealed that the aflatoxin measurements between the two agencies were significantly ( $p < 0.01$ ) related, with a correlation coefficient of  $r = 0.80$ . The outbound trucks sampled by OTSC were subjected to a hierarchical analysis to derive grain elevator, grain bin, truck-to-truck and intra-truck variance components. The variance was partitioned as follows: grain elevator variance (1.9%), bin variance (65.8%), truck variance (9.1%) and the residual error (23%) representing intra-truck aflatoxin variability. This study documents that the negotiated sampling plan provided regulators the ability to detect and isolate grain unfit for commerce.

## 1. Introduction

Aflatoxin is a group I carcinogen as defined by the International Agency for Research on Cancer (IARC, 1993) and may occur in susceptible crops including cereals, oilseeds, and tree nuts. In the United States (US), there are no regulatory limits prescribed by the Food and Drug Administration

(FDA), however, the agency has issued a Compliance Policy Guide (CPG Sec. 683.100) listing aflatoxin action levels, which the US food and feed industry follow (FDA, 1994). In particular, maize containing  $>20$  µg/kg (commonly expressed as parts per billion) may be channeled to animal feed as follows: 300 µg/kg for finishing beef

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cattle; 200 for finishing swine of 100 pounds or greater; 100 µg/kg for breeding beef cattle, breeding swine, or mature poultry; 20 µg/kg for all other animals. In Texas, grain and oilseeds containing more than 20 µg/kg aflatoxin and 5 µg/g fumonisin are defined as adulterated by the Texas Commercial Feed Control Act section 141.002(c). Regulatory oversight for the distribution of adulterated grain and oilseed (defined as commercial feed) is provided by the Texas Feed and Fertilizer Control Service, which is part of the Office of the Texas State Chemist (OTSC). If aflatoxin concentrations exceed 300 µg/kg, companies must submit either a blending or disposition plan as required in the Texas Commercial Feed Rules (OTSC, 2011).

Growers insure their crop for yield and quality loss through the US Department of Agriculture (USDA) crop insurance program administered through the Risk Management Agency (RMA). To collect aflatoxin loss indemnification, grain samples must be collected using procedures outlined in the RMA Loss Adjustment Manual (LAM) and submitted to an approved laboratory (USDA, 2012). For maize exceeding 300 µg/kg aflatoxin “a claim cannot be completed until such production (including unharvested production) is sold, fed, used, or destroyed.”

In 2009, a grain company in Texas filed for bankruptcy with the Texas Department of Agriculture Warehouse Examination Division. The bankruptcy file contained the crop insurance aflatoxin results listed by producer but did not specify the particular grain elevator or bin in which the grain was stored. The aflatoxin measurements were performed by three RMA approved laboratories (2010), two of which were approved Grain Inspection, Packers and Stockyard Administration (GIPSA) Official Inspection Agencies (OIA) and the third was a private grain exchange approved by RMA. Review of the bankruptcy file by OTSC management caused Texas feed control officials sufficient concern that they seized all grain within the seven grain elevators and required aflatoxin contaminated maize be managed through a prescribed procedure

outlined in Feed Industry Memorandum 5-12 (OTSC, 2010). At the time of the bankruptcy filing, OTSC already had seized three of the 38 bins containing maize. OTSC obtained the bankruptcy documents through a formal request under the state’s freedom of information regulations from the Texas Department of Agriculture.

In the absence of information documenting individual grain lot placement by grain elevator and bin, the Texas State Chemist and bankruptcy court appointed attorney negotiated terms for grain sampling and testing to assess aflatoxin levels of contamination. In particular, the settlement required that a GIPSA OIA perform official sampling and testing of maize using official protocol (USDA-GIPSA, 2009a). Under this agreement, the first five truckloads from each bin were sampled and tested for aflatoxin. If the aflatoxin results for all five truck loads were 300 µg/kg or less, maize could be distributed into interstate commerce per FDA’s CPG Sec. 683.100 and labeling approved by OTSC. Maize testing from 301 to 500 µg/kg would be subject to a blend plan and could be distributed within the state of Texas. Maize containing greater than 500 µg/kg did not enter commerce and a disposition plan was required (OTSC, 2010). Because the maize containing >300 µg/kg aflatoxin was grown, stored, blended, and distributed within the state of Texas there was no involvement by, nor did FDA have any legal jurisdiction over this activity (FDA, 2012).

To evaluate the efficacy of this strategy and the variance structure of contaminated maize within a large commercial grain elevator complex, OTSC also sampled truckloads of maize and tested these samples for aflatoxin. In a previous study, Johansson et al (2000) quantified the difference sources of variation associated with measuring aflatoxin into sampling, sample preparation, and analytical variation. They reported that 78% of the total variability (CV=82.9%) was attributed to sampling, 28% of the total variation (CV=37.5%) was attributed to sample preparation and 2% of the total variation

(CV=10.7%) was attributed by sample analysis. The variance partitioning described by Johansson et al was intended to assess the impact of GIPSA sampling procedures on the source of total variability in aflatoxin and did not consider the impact of different testing procedures, different analysts, nor did it consider variance within a commercial scale storage and handling system. Thus, this study was performed to quantify aflatoxin variance components in a commercial grain storage to assist regulatory risk managers evaluate the effectiveness of their sampling scheme and regulatory oversight to protect animal, human, and market health.

## 2. Materials and methods

### 2.1. Sampling

The sampling methodology was designed to test the hypothesis; did the negotiated disposition plan between OTSC and the bankruptcy attorney manage aflatoxin risk? The sample frame for this study was derived from the Texas Department of Agriculture Commercial Warehouse Division audit report that listed maize stored at seven commercial grain elevator locations in 38 individual bins. Truckloads of maize reclaimed and shipped during May 18, 2010 to July 22, 2010 were monitored by an OIA per negotiations between the court appointed bankruptcy attorney and the Texas State Chemist. The negotiated settlement involved sampling the first 5 truckloads from every bin containing maize. Bins with the first five loads testing  $\leq 300$   $\mu\text{g/kg}$  required no further sampling, bins with maize testing  $>300$  to  $\leq 500$   $\mu\text{g/kg}$  were subject to a blending plan with further testing, and bins with maize  $>500$   $\mu\text{g/kg}$  aflatoxin required that all truckloads be sampled and tested for aflatoxin. The GIPSA sampling procedure involves collection of 7 hand probes using a pattern specified in the Federal Grain Inspection Service (FGIS) aflatoxin handbook (USDA-GIPSA, 1995). In total, 301 outbound trucks were subjected to sampling by the OIA in

conformance with the disposition plan. The OTSC sampling study included 64 of the 301 outbound trucks. These trucks originated from 30 bins at 6 grain elevators, ranging from 1 to 3 truckloads per bin. An OTSC field investigator sampled trucks using GIPSA protocol and, in addition, three single probes per truck were collected and identity retained for a variance component analysis.

Sample preparation by the OIA involved grinding the maize using a Romer<sup>®</sup> Labs, Inc. mill. Procedures outlined in the GIPSA Aflatoxin Handbook for the Vicam AflaTest<sup>®</sup> testing platform (USDA-GIPSA, 2009b) were followed by the OIA for aflatoxin analysis that was performed onsite. OTSC samples were shipped to the College Station headquarters using chain-of-custody protocol.

### 2.2. Sample Preparation

The OTSC maize samples were ground using a Romer<sup>®</sup> Labs, Inc. mill model 2A (Romer<sup>®</sup> Labs, Inc., Washington, MO) then subjected to a second grind by Retsch<sup>®</sup> Ultra Centrifugal Mill ZM 200 (Haan, German) using a 0.75 mm screen. The aflatoxin testing procedures used by OTSC conforms to the AOAC methodology for high performance liquid chromatography (HPLC), UV and fluorescence detection (AOAC, 2005). Certified AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> purchased from Romer<sup>®</sup> Labs, Inc.-Biopure (Tulln, Austria) and were used as standards. All solutions are made with HPLC-grade solvents and reagent grade materials unless otherwise noted. The concentration of AB<sub>1</sub> and AFG<sub>1</sub> standard is 2  $\mu\text{g/mL}$  in 5 mL acetonitrile. The concentration of AFB<sub>2</sub> and AFG<sub>2</sub> standard is 0.5  $\mu\text{g/mL}$  in 5 mL acetonitrile.

### 2.3. Statistical analyses

The descriptive statistics function in Microsoft Excel<sup>®</sup> were used to analyze OIA results for incoming aflatoxin analyses performed for crop insurance by three RMA approved laboratories and for the outgoing truck samples analyzed by the OIA. These results were compared. A

correlation analysis was performed to compare GIPSA-OIA and OTSC truck analysis results using the Microsoft Excel<sup>®</sup> charting and trend line function. The variance structure analysis performed using OTSC individual probe and composite probe data were performed using the NESTED and GLM procedures of SAS<sup>®</sup> software (2009). Since the data were unbalanced, the GLM procedure was performed with the NESTED option. The NESTED procedure may produce unbiased estimates for the variance components in an unbalanced design, but F-tests in the analysis of variance are not presented. Therefore, P-values corresponding to F-statistics for each level of variable (facility, bin, and truck) were obtained using the GLM procedure.

### 3. Results and discussion

#### 3.1. Comparison of incoming and outgoing trucks

The paucity of company records prevented a comparison of incoming and outbound trucks containing maize grain by bin or elevator. However, a comparison of the average aflatoxin measures for all 551 incoming trucks and 301 outbound trucks were 373 µg/kg and 376 µg/kg, respectively (Table 1). The descriptive statistics reported in Table 1 indicate a lighter tail for both populations (heavy concentration around the mean) as evidenced by a kurtosis value of 1.9 and 0.2 for the incoming and outgoing grain and a positive skewness. The positive skewness results from some highly contaminated samples, specifically, 1900 µg/kg and 1160 µg/kg aflatoxin for incoming and outbound grain, respectively.

A comparison of the incoming and outbound maize samples reveals that approximately 45% of the incoming and 35% of the outbound grain were above 300 µg/kg. The incoming percentage was calculated as a percent of total crop insurance samples. The outbound maize sample results were calculated using truck scale ticket weights. For outbound shipments, the first five truckloads for every grain bin were

**Table 1**

Descriptive statistics for incoming and outgoing maize samples contaminated with aflatoxins.

Aflatoxin descriptive statistics	Incoming	Outgoing
Count	551.0	301.0
Mean, µg/kg	373.4	376.2
Percent ≤300 µg/kg	54.8	65.1
Percent >300 µg/kg	45.2	34.9
Standard error, µg/kg	16.4	12.9
Standard deviation, µg/kg	383.8	223.7
Median, µg/kg	260.0	360.0
Mode, µg/kg	0.0	600.0
Kurtosis	1.9	0.2
Skewness	1.4	0.6
Range, µg/kg	1900.0	1153.8
Minimum, µg/kg	0	6.2
Maximum, µg/kg	1900.0	1160.0
Confidence level (95.0%)	32.1	25.4

evaluated for aflatoxin. Those bins with samples above 300 µg/kg aflatoxin were tested in their entirety. In total, 15 of the 38 bins displayed aflatoxin contamination at or below 300 µg/kg aflatoxin based on measurement of the first 5 trucks reclaimed from the grain bin. As prescribed in the negotiated settlement with the bankruptcy attorney, 229 trucks of reclaimed maize were not tested for aflatoxin from these bins.

Maize was stored in bins constructed of either corrugated steel or concrete. The grain flow during reclaim differs between these types of storage facilities due, in part, to the bin dimensions. Corrugated steel bins possess a higher diameter to height ratio and funnel flow occurs during bin discharge, leading to an increased amount of grain mixing. Concrete silos possess a lower diameter to height ratio and mass grain flow occurs during grain discharge resulting in less grain mixing (Reed, 2006). Ten of the 38 bins were corrugated steel; of these only 4 yielded aflatoxin tests >300 µg/kg. In contrast, 19 of the 28 concrete silos yielded aflatoxin test results >300 µg/kg. The strategy of testing the first 5 trucks appeared most effective in isolating maize

contaminated with aflatoxin >300 µg/kg from concrete silos. A paucity of records and overall business practices by the firm that lead to the bankruptcy prevents any further interpretation of these data.

The negotiated procedure of testing of the first five truckloads of maize per bin resulted in the identification and isolation of approximately 1900 tons of contaminated maize containing >500 µg/kg aflatoxin. The trucks of maize were disposed of per a plan provided by the bankruptcy lawyer and the process was observed by an OTSC field investigator.

### 3.2. Interagency comparison

The descriptive statistics for the interagency comparison of 64 trucks of maize sampled and evaluated by both GIPSA-OIA and OTSC for aflatoxin are presented in Table 2. The means values were 383 µg/kg aflatoxin and 453 µg/kg aflatoxin for the OIA and OTSC, respectively. Both agencies utilized a 7 probe composite sample conforming to the GIPSA aflatoxin handbook and comminuted samples using a Romer® Labs, Inc. mill. OTSC further grinds samples using a Retch® mill to increase the number of particles in a 50 gram sample to improve sample test reproducibility. FGIS requires that a minimum of 60% of the ground material pass through a 20 mesh sieve while OTSC grinding results in 100% passing through a 20 mesh sieve. OTSC utilizes the AOAC method performed on HPLC for aflatoxin analysis.

The OIA and OTSC results were significantly related ( $P < 0.01$ ) with the correlation coefficient  $r = 0.80$  (Figure 1). Both population distributions displayed a positive skewness with maximum values of 1,080 µg/kg aflatoxin and 1,460 µg/kg aflatoxin for the OIA and OTSC, respectively. The average deviation between the OIA and OTSC was 30%. This difference could be attributed to the grinding

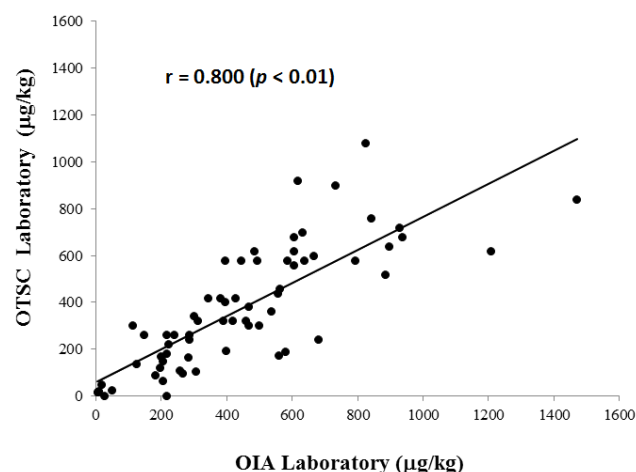
**Table 2**  
Descriptive statistics for comparison of aflatoxins evaluated by GIPSA-OIA and OTSC.

Aflatoxin descriptive statistics	GIPSA-OIA <sup>a</sup>	OTSC <sup>b</sup>
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Count	64.0	64.0
Mean, µg/kg	383.8	453.5
Standard error, µg/kg	31.6	36.4
Standard deviation, µg/kg	252.9	290.9
Median, µg/kg	320.0	422.5
Mode, µg/kg	580.0	215.0
Kurtosis	-0.3	1.5
Skewness	0.6	0.9
Range, µg/kg	1064.8	1465.0
Minimum, µg/kg	15.2	5.0
Maximum, µg/kg	1080.0	1470.0
Confidence level (95.0%)	63.2	72.7

<sup>a</sup> Grain Inspection, Packers and Stockyard Administration (GIPSA) Official Inspection Agency (OIA)

<sup>b</sup> Office of the Texas State Chemist (OTSC)



**Fig. 1.** A scatter plot of GIPSA-OIA laboratory versus OTSC laboratory determined aflatoxin values.

difference, in which the more finely ground material by OTSC yields more particles per 50 grams and thus, a more representative sample, compared to the OIA. Additionally, OTSC runs two control samples for every batch of analyses to avoid analytical bias and analyzes samples using an AOACI approved methodology. At the time of this study, GIPSA had only approved field tests for measuring

aflatoxin concentrations in maize at a 100 µg/kg maximum (GIPSA, 2009a), no other validation of these tests for >100 µg/kg had been performed by a competent authority, and OIAs were not required to run controls (maize samples with known aflatoxin levels) as part of their quality assurance program.

### 3.3. Variance components of aflatoxin distribution

The variance component analysis for an unbalanced nested design partitioned aflatoxin variances into facility, bin, truck, and error (Table 3). The total variance of aflatoxin concentration mainly consists of bin variance and sampling error. Bin variance represented 66% of the total variance and is attributable to the variations of aflatoxin concentration of incoming maize and grain flow properties within a grain bin. Most commercial grain elevators blend maize from multiple bins during reclaim to overcome a uniform flow of grain, which typically occurs if the silo possesses a greater height than width (e.g., 40 meters tall by 6 meters diameter). In this study, grain from individual bins were reclaimed and discharged into trucks due to the terms of the

bankruptcy settlement. The sampling error characterizes the intra-truck variation captured through the individual probes of maize. The coefficient of variation among individual probes within a truck ranged between 0% and 92.2% with an average CV of 28.4% across 64 trucks. A hierarchical variance component analysis drops the lowest treatment of the design and this variance, along with other experiment error, is captured within the error term.

The unbalanced nested experimental design occurred due to the uneven number of grain bins containing maize at each commercial elevator. The study targeted a minimum of three bins per elevator, but only one bin contained maize at one location and two bins contained maize at two other locations.

In this study, two separate samples were collected from 30 ton truckloads of maize that were analyzed in two government laboratories. Results from this study document that a 30% average deviation between these two official testing agencies and provide an initial benchmark for what to expect in terms of actual variation when official sampling and testing procedures are followed by competent authorities based on current aflatoxin testing technologies.

**Table 3**

Variance of aflatoxin distribution throughout grain facility and transportation<sup>a</sup>

Variance source	F-value	P-value	Variance component	Percent (%) of total variance
Total			91,357	100.0
Facility	1.06	0.4006	1,724	1.9
Bin	9.81	< 0.0001	60,130	65.8
Truck	2.14	0.0007	8,290	9.1
Error			21,212	23.2

<sup>a</sup> Values presented in this table are obtained from both NESTED and GML procedures.

### 3.4. Risk management implication

This study documents the aflatoxin variance structure within a commercial grain elevator and

establishes a benchmark for variability that can be applied to grain industry best practices for aflatoxin risk management. The mean, kurtosis coefficient and skewness coefficient documents a similar distribution of aflatoxin detected among incoming and outbound trucks. The sampling of 5 trucks per bin was performed in absence of records documenting placement of aflatoxin contaminated maize by grain elevator or bin. While testing every load may be desirable by a regulatory risk manager, a negotiated sampling plan between the Texas State Chemist and bankruptcy attorney mitigated the potential harms associated with judicial settlement that may have prevented outbound sampling and testing of aflatoxin. The sampling strategy prevented 1900

tons of maize containing greater than 500  $\mu\text{g/kg}$  from entering commerce.

The OTSC manages aflatoxin risk based on the presence or absence of records documenting that a firm has implemented a sample and analytical plan for maize. In the presence of sample analytical data, Table 4 defines the criteria for a blending plan for maize that is adulterated with  $>300 \mu\text{g/kg}$  and  $\leq 500 \mu\text{g/kg}$  aflatoxin and Table 5 defines the criteria for a disposition plan for maize that is adulterated with  $>500 \mu\text{g/kg}$  aflatoxin. The sampling of 5 outbound loads of maize per bin has since been adopted by the OTSC for managing aflatoxin risk in those instances where there is an absence of sample analytical data.

**Table 4**

Blending plan for corn adulterated with  $>300 \mu\text{g/kg}$  and up to  $500 \mu\text{g/kg}$  aflatoxin.

The Texas Administrative Code, Title 4, Chapter 61, Subchapter H Adulterants, 61.61 (a) (6) and the Office of the Texas State Chemist (OTSC) states that grain containing  $>300$  to  $500 \mu\text{g/kg}$  aflatoxin requires a blending permit issued by the Office of the Texas State Chemist. Guidance document 5-12 describes elements in the plan that include corn with aflatoxin levels from  $300$  to  $500 \mu\text{g/kg}$  aflatoxin will be blended with corn containing greater than  $20 \mu\text{g/kg}$  aflatoxin to a level under  $200 \mu\text{g/kg}$  aflatoxin. The firm distributing the product must be licensed with the OTSC and a blending plan must be approved by the OTSC for each crop year prior to distribution.

*The blending plan must include:*

1. Method for blending.
2. The sampling scheme and testing procedures must conform to the One Sample Strategy Handbook (OTSC, 2013).
3. Frequency of sampling (e.g. firm will sample every 5<sup>th</sup> load involved in the blending, etc.).
4. The plan for reblending or disposition of blended corn exceeding  $200 \mu\text{g/kg}$  aflatoxin.
5. The plan for disposition of blended corn exceeding  $500 \mu\text{g/kg}$  aflatoxin and not allowed to enter commerce (e.g. landfill, plowing, etc.).
6. A copy of the label for the blended corn.
7. A written commitment to provide the aflatoxin test results, the final destination, any associated firm and/or broker, and the amount of blended corn distributed to the OTSC.
8. A written commitment by the firm to retain the records of distribution and aflatoxin test results of blended corn for two years.
9. A written commitment that the blended corn will not be shipped in interstate commerce.
10. A written commitment that the blended corn will only be distributed for finishing beef cattle in confinement for slaughter.

**Table 5**

Blending plan for corn adulterated with >300 µg/kg and up to 500 µg/kg aflatoxin.

The Texas Administrative Code, Title 4, Chapter 61, Subchapter H Adulterants, 61.61 (a) (6) and the Office of the Texas State Chemist (OTSC) requires a record of disposition for corn containing greater than 500 µg/kg aflatoxin and does not allow the product to enter commerce. A record of disposition must be submitted for the quantity of corn adulterated with greater than 500 µg/kg aflatoxin.

*The disposition plan must include:*

1. Testing of every load reclaimed from the grain storage bin.
2. The sampling scheme and testing procedures must conform to the One Sample Strategy Handbook (OTSC, 2013).
3. The method of disposition (e.g., landfill, plowing, etc.).
4. The amount of corn to be disposed.
5. The location of the disposition.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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