JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8481–8489 8481
DOI:10.1021/jf1018356

Effect of Sample Size in the Evaluation of "In-Field" Sampling Plans for Aflatoxin B_1 Determination in Corn

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Use of proper sampling methods throughout the agri-food chain is crucial when it comes to effectively detecting contaminants in foods and feeds. The objective of the study was to estimate the performance of sampling plan designs to determine aflatoxin B_1 (AFB_1) contamination in corn fields. A total of 840 ears were selected from a corn field suspected of being contaminated with aflatoxin. The mean and variance among the aflatoxin values for each ear were 10.6 μg/kg and 2233.3, respectively. The variability and confidence intervals associated with sample means of a given size could be predicted using an equation associated with the normal distribution. Sample sizes of 248 and 674 ears would be required to estimate the true field concentration of 10.6 μg/kg within $\pm$50 and $\pm$30%, respectively. Using the distribution information from the study, operating characteristic curves were developed to show the performance of various sampling plan designs.

KEYWORDS: Aflatoxin; sampling; field; corn; ear; Monte Carlo

INTRODUCTION

Food safety issues have recently been treated at the international level with a homogeneous trend aimed at guaranteeing as much as possible the protection of animal and public health from farm to fork by the implementation of preventive actions based upon the adoption of good agricultural practices as a starting point (*1*). In this context, European Regulation 178/2002 (*2*) stated that all of the stakeholders involved in the agri-food chain are directly responsible for the safety of raw agricultural commodities including food and feed. In this scenario, different tools can be taken into consideration, but undoubtedly the sampling step is one of the most crucial and sometimes the most underestimated part of the multifaceted and complex activities aimed at addressing and managing food safety issues. In practice, the overall objective of a good sampling program is to provide reliable sample test results representing the basis for "fit for purpose" investigations (*3*). Therefore, the most important reason for collecting food samples for the investigation of contaminants such as mycotoxins is to increase as much as possible the effectiveness of methods used to protect consumer health and verify the compliance of food and feed with acceptable safety standards. This issue is crucial for all of the involved stakeholders.

The primary objective of a food safety program is to guarantee the right to health as a non-negotiable issue. However, differences in food safety approaches and regulations worldwide can significantly interfere with marketing of food products, creating discrepancies in food safety standards and serious legal and economic obstacles to trade and restricting competition among trading enterprises. In addition, the appropriateness of the final information to be used in risk analysis depends on the reliability and coherency not only of the experimental data but also of the related metadata. (Metadata means "data about data" or the structured information that describes, explains, locates, or otherwise makes easier to use or manage the experimental data.) As far as sampling is concerned, the rule of the four Ws (why, what, when, and where) should be adopted throughout the overall agri-food chain to put in place the most convenient strategy for detecting contaminants in food and feed (*3*).

In this context, the advantage of sampling agricultural commodities in fields has not been investigated as extensively as sampling commodities for contaminants after harvesting or processing. The estimation of the true aflatoxin concentration in a field is a complex issue, first due to the heterogeneity of the aflatoxin distribution among units of concern in the field and second because of the large variability among unit concentration values, which is made more complicated by the period of maturation of the crop. A heterogeneous distribution of aflatoxin B_1 (AFB_1) among members of a population is characterized by a distribution with a small percentage of highly contaminated members in comparison to the rest of the population with little or no contamination. For this reason, an Italian project named AFLARID was finalized with the aim to define a sampling plan to determine the AFB_1 concentration among individual corn ears in a corn field. In particular, the purpose of this study was to determine the effect of sample size (number of ears) on the variability among sample

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Published on Web 07/07/2010

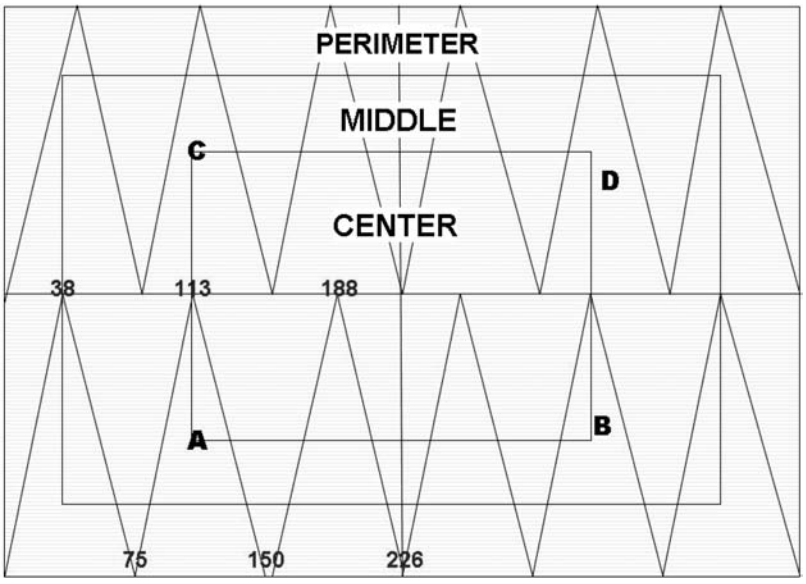

Figure 1. Pattern of sampling used in the study. Zones and areas of the field.

concentrations of a given size. With this information, sample size can be recommended to farmers that will reduce the risk of misclassifying fields as acceptable or unacceptable when it comes to aflatoxin contamination levels.

MATERIALS AND METHODS

In-Field Sampling Plan. The first step was to choose a corn field suspected of AFB_1 contamination. A field was chosen that had approximately 83000 plants. Eight hundred and forty ears were selected from the field according to ISO 2859 (*4*). It was assumed that 840 ears was large enough to have an accurate estimation of the true AFB_1 contamination of the field. The field was divided into four areas, and the ears were taken from each area following a triangular-shaped model where 37 or 38 ears were taken from every side of the triangle. This pattern allowed ears to be taken from both the central and peripheral zones of the field (**Figure 1**).

AFB_1 Detection. AFB_1 was determined in each ear of corn using an analytical method developed previously by Brera et al. (*5*). Basically, corn kernels from each ear (200 g on average) were thoroughly ground with a ZM 200 ultracentrifugal mill (Retsch, Haan, Germany) mill using a screen with 0.5 mm diameter openings. From the ground corn, a 25 g test portion was extracted with methanol/water (80:20) (Carlo Erba, Vigevano, Italy); the extract was filtered, diluted with a phosphate-buffered saline solution, filtered on a microfiber glass filter, and applied to an Easi-Extract Aflatoxins RP70N immunoaffinity column (R-Biopharm Rhône, Glasgow, Scotland). The column used was a 250 mm × 4.6 mm i.d., 5 μm, Symmetry RP-18 (Waters, Milford, MA); it was washed with deionized water (Millipore, Billerica, MA) to remove interfering compounds, and the purified AFB_1 was eluted with methanol (Carlo Erba, Vigevano, Italy). AFB_1 was separated and determined by reversed-phase LC with fluorescence detection after postcolumn derivatization (Giasco, Great Dunmow, U.K.).

Using the Normal Distribution To Determine the Effect of Sample Size on Precision. The law of large numbers (*6*) indicates that regardless of the aflatoxin distribution among individual units in the population (corn ears in the field), as the size of the sample taken from the population increases, the distribution among sample means of a given size approaches a normal distribution. Under the assumption of normality of the distribution of sample means, the sample size (n) is computed, for a certain precision, according to eq 1 (*7*, *8*)

$$n = \frac{v_1}{\Delta^2} \times Z^2 \qquad (1)$$

where v_1 = variance among individual units, Δ = deviation of sample mean about the population mean, and Z = the critical value of normal distribution at 95% level of confidence.

This classical approach to calculating a sample size for a given precision is based on the assumption of normality of the distribution of the sampling means.

Monte Carlo Approach To Determine the Effect of Sample Size on Precision. The Monte Carlo approach (*9*) was used to sample theoretically the field. to evaluate the effect of the sample size on the normality assumption, and to determine the variability (precision) among sample means of size n when the true field AFB_1 concentration was estimated. This approach is an iterative, nonparametrical technique that does not need to make an assumption (as in the classical approach) about the aflatoxin distribution among the ears of corn in the field (*9*). The sole assumption is that the observed aflatoxin distribution among the 840 ears is considered to be the "true" aflatoxin distribution among individual ears of corn in the field.

With the Monte Carlo method, a uniformly distributed random number between 0 and 1 is generated to simulate the random selection of an ear of corn from the cumulative aflatoxin distribution among the 840 ears of corn. Generating n random values is equivalent to selecting a sample of n ears of corn from the field. To study the effects of sample size (number of ears) on the variability (precision) among samples of size n, samples of 5, 10, 20, 40, 80, 160, and 320 ears were selected form the aflatoxin distribution among the 840 ears. Each sample size was selected 10000 times, and several simple statistics and confidence limits were computed.

RESULTS AND DISCUSSION

Descriptive Analysis of AFB_1 Contamination of the 840 Ears. Simple statistics such as the mean and variance among the aflatoxin test results for the 840 individual ears have been computed. The average AFB_1 concentration among the 840 values was 10.6 μg/kg, the median was 0.03 μg/kg (LOQ/2), the variance was 2233.2, and the coefficient of variation was 446.5%. The simple statistics indicate that the variability among the 840 AFB_1 values for each individual ear is large, and because the mean (10.6 μg/kg) is greater than the median (LOQ/2), the AFB_1 distribution among the 840 ear values is positively skewed. These features of the aflatoxin distribution resulted from the presence of a large percentage of ears with little to no aflatoxin contamination in comparison with very few ears with high levels of contamination. In particular, five ears measured above 300 μg/kg (369, 400, 542, 600, 662 μg/kg). In 12% of ears, the AFB_1 levels were > 5 μg/kg, which is the legal limit according to EC Regulation 1881/2006 (*10*) for food. A total of 10% of ears had concentrations > 20 μg/kg, which is the legal limit for feed (Directive EC/2003/100) (*11*).

Analysis by Areas of the Field. Each of the 840 AFB_1 values are identified by one of the four areas (A, B, C, D) of the field from which each ear was taken to determine if the mean AFB_1 contamination level was different among the four areas (**Figure 1**). Comparing the mean AFB_1 contamination level for each of the four areas showed similar average contamination values among all four areas. The mean AFB_1 contamination level for area B had both the lowest variance and average contamination level of the four areas (**Table 1**). The GENMOD procedure in SAS (SAS Institute Inc., Cary, NC) was used (*12*, *13*) to test the null hypothesis of homogeneity of the mean contamination in the four areas. The output of this test indicated that the null hypothesis could not be rejected at the 95% confidence level.

Table 1. Descriptive Analysis of AFB_1 Contamination per Area of the Field

area	frequency	percentage	av contamination (μg/kg)	SD	variance
A	215	25.6	11.0	52.4	2743.8
B	199	23.7	8.2	27.6	763.3
C	215	25.6	10.6	38.2	1460.5
D	211	25.1	12.4	62.5	3908.7
total	840	100.0	10.6	47.2	2233.2

Table 2. Descriptive Analysis of AFB_1 Contamination per Zone of the Field

zone	frequency	percentage	av contamination (μg/kg)	SD	variance
perimeter	368	43.8	5.9	22.3	497.0
middle	330	39.3	17.3	69.2	4793.1
center	142	16.9	7.0	25.2	635.1
total	840	100.0	10.6	47.2	2233.2

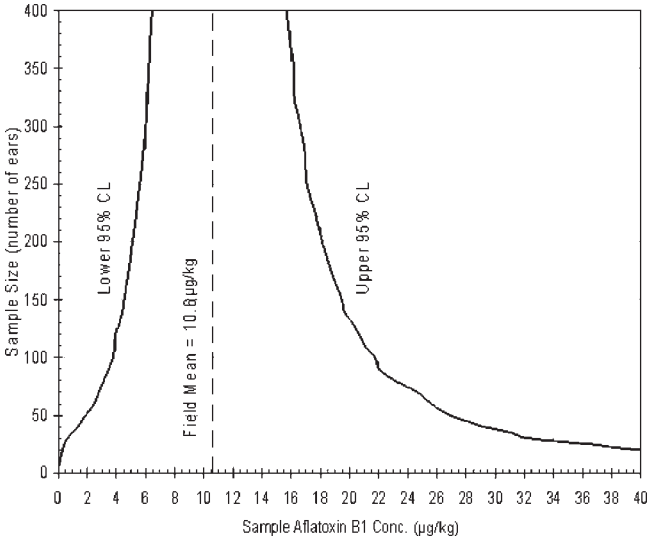

Figure 2. AFB_1 distribution among 840 individual ears of corn. Average AFB_1 concentration among all 840 ears is 10.6 μg/kg. The maximum ear concentration was 662 μg/kg AFB_1 and is not shown.

Aflatoxin Distribution by Zones of the Field. To determine if the AFB_1 contamination can be different in the center of the field compared to the perimeter of the field, the entire field was divided into three concentric zones: center of the field (center), intermediate zone of the field (middle), and perimeter zone (perimeter). A graphical representation of the three zones is shown in **Figure 1**. Each ear AFB_1 value was classified into one of the three concentric zones of the field from which the ear was taken to determine if there are differences in the AFB_1 contamination level of the three concentric zones (**Figure 1**). Comparing the mean AFB_1 contamination levels in **Table 2** shows that the middle zone had the highest average contamination value. The GENMOD procedure in SAS (SAS Institute Inc.) indicated that the difference between mean contamination level among the middle zone and the others zones of the field is statistically significant (p = 0.0011) (*12*, *13*).

Location of Contaminated Ears in the Field. With the aim of identifying potential clustering points where hot spots of contamination could be concentrated, the location of the contaminated ears in the field was evaluated. The highly contaminated ears were distributed throughout all three zones. However, five ears with the most contamination were all located in the middle zone. This resulted in the middle zone having the highest AFB_1 contamination level in comparison to the other zones.

Monte Carlo Sampling. For each of seven sample sizes (5, 10, 20, 40, 80, 160, and 320 ears), the 95% interval of confidence (IC) among the 10000 sample concentration values was determined (**Figure 2** and **Table 3**). As expected, the average AFB_1 among the 10000 sample means was similar for all seven sample sizes. However, as the sample size increases from 5 to 320 ears, the median and average of the aflatoxin distribution among sample means became similar. As sample size increases, the median approaches the average and the distribution among sample concentration becomes more symmetrical or approaches a normal distribution. The variance among sample means (fourth line of **Table 3**) decreases as sample size increases, and the confidence interval becomes tighter (line seven of **Table 3**). In fact, Monte Carlo results followed those predicted by theory, which states that if sample size is doubled, the variance among sample means is reduced by half.

The upper (97.5%) and lower (2.5%) probabilities associated with 95% confidence limits in **Table 3** are plotted in **Figure 2**. The plot was developed by interpolating between the 95% confidence limit computed for samples of various size (5, 10, 20, 40, 80, 160, 320 ears) in **Table 3**. **Figure 2** graphically describes how the confidence interval of the distribution of sample mean decreases as the sample size increases, reflecting a reduction in variance as sample size increases. Moreover, the graph in **Figure 2** shows that the 95% confidence intervals are not symmetrical, especially for small sample sizes about the true field concentration (10.6 μg/kg), until sample sizes exceed 200−300 ears. Through the use of the Monte Carlo method, the precision for different sample sizes was computed without having to assume the distribution among

Table 3. Precision and Distribution Estimates among Sample Test Results for Samples of Various Sizes Taken from All Ears (N = 840; Monte Carlo Approach)

	sample size in number of ears						
	5 ears	10 ears	20 ears	40 ears	80 ears	160 ears	320 ears
no. of samples analyzed	10000	10000	10000	10000	10000	10000	10000
av of sample means of AFB_1 in all samples (μg/kg)	10.8	10.8	10.7	10.7	10.7	10.6	10.6
median of sample means of AFB_1 in all samples (μg/kg)	1.0	5.9	7.8	8.6	9.6	10.2	10.4
variance of sample means of AFB_1 in all samples	447.9	225.0	113.3	56.3	28.3	14.1	7.1
min AFB_1 of sample means of AFB_1 in all samples (μg/kg)	0.0	0.0	0.0	0.2	0.7	2.1	3.8
max AFB_1 of sample means of AFB_1 in all samples (μg/kg)	254.0	152.0	40.3	61.2	40.0	30.8	26.9
CI 95% of sample means of AFB_1 in all samples (μg/kg)	0.0−79.8	0.1−60.8	0.3−40.4	1.4−29.8	3.2−23.5	4.7−19.2	6.1−16.4

Table 4. Population Sample Size with 50, 30, and 10% of Precision[a]

mean (μg/kg)	IC (95%) for a precision of 50% (μg/kg)	sample size with 50% of precision	IC (95%) for a precision of 30% (μg/kg)	sample size with 30% of precision	IC (95%) for a precision of 10% (μg/kg)	sample size with 10% of precision
6.00	3.00−9.00	219	4.20−7.80	608	5.40−6.60	5468
7.00	3.50−10.5	226	4.90−9.10	627	6.30−7.70	5643
8.00	4.00−12.00	232	5.60−10.40	644	7.20−8.80	5799
9.00	4.50−13.50	238	6.30−11.70	660	8.10−9.90	5940
10.00	5.00−15.00	243	7.00−13.00	674	9.00−11.00	6069
11.00	5.50−16.50	248	7.70−14.30	688	9.90−12.10	6188
12.00	6.00−18.00	252	8.40−15.60	700	10.80−13.20	6299
13.00	6.50−19.50	256	9.10−16.90	711	11.70−14.30	6403
14.00	7.00−21.00	260	9.80−18.20	722	12.60−15.40	6501
15.00	7.50−22.50	264	10.50−19.50	733	13.50−16.50	6593
16.00	8.00−24.00	267	11.20−20.80	742	14.40−17.60	6680
17.00	8.50−25.50	271	11.90−22.10	751	15.30−18.70	6763

[a] Variance = $9.872 \times \text{mean}^{2.2042}$.

sample means is normal. However, **Table 3** and **Figure 2** indicate that for sample sizes above about 200 ears, the distribution among sample concentrations can be approximated with the normal distribution, and eq 1 can be used to compute the effects of sample size on precision.

Classical Approach. Monte Carlo results (**Table 3** and **Figure 2**) indicate that for sample sizes above about 200 ears, the distribution among sample concentrations can be approximated with the normal distribution. Under the assumption of normality of the aflatoxin distribution of sample means, for sample sizes above 200 ears, the sample size n was computed, for a certain precision using eq 1. Despite a strongly skewed distribution among the aflatoxin concentrations of the 840 individual ears, the classical approach that uses normality assumptions (eq 1) can be used to compute the effect of sample sizes above 200−300 ears on reducing the variability or increasing the precision associated with estimating the true field aflatoxin concentration.

Variance as a Function of the Aflatoxin Concentration. The above discussion on the effect of sample size on precision is specific to the field sampled in this study with a mean concentration of 10.6 μg/kg and a variance of 2233.2. Previous studies (*14*) have indicated that sampling variance is a function of the aflatoxin concentration in a field. To formulate the function between the variance and mean of the contamination of AFB_1 of the field, it is necessary to have data from different fields. Because of limited resources, data from other fields contaminated by AFB_1 were not available. Dividing the field into four areas (**Figure 1** or A, B, C, and D) cannot help given that the areas have similar mean contamination levels. However, mean and variance data from the concentric zones of the field were used (**Figure 1**) to develop a functional relationship between mean and variance because the three zones had different aflatoxin concentrations. The three concentric zones were considered as three different fields with different variance and mean concentrations. Whitaker et al. (*14*) demonstrated that sampling variance increased with aflatoxin concentration and could be approximated by a linear regression in the log−log scale

$$\text{variance} = 9.872 \times \text{mean}^{2.2042} \quad (2)$$

where $R^2 = 0.9788$.

Equation 2 was used to calculate the variance expected for different aflatoxin concentrations for different sample sizes. Results are shown in **Table 4**. All of the sample sizes computed are within a range of 219−271 ears for ±50% precision; therefore, a conservative sample size of 271 ears was chosen for the sample size that can be used to estimate field concentrations in the range of interest (6.00−17.00 μg/kg). A precision of ±50% about the true field concentration may be considered too large, but a sample size of 271 ears from field concentrations of 13.0 μg/kg or less will not be rejected in most cases using the 20.0 μg/kg regulatory limit (*11*). Samples of 271 ears will test below 19.5 μg/kg when taken from fields with ≤13.0 μg/kg aflatoxin concentration. For an estimation of the field concentration with a precision of ±30%, a sample size of 751 can be used to estimate field contamination levels between 6.00 and 17.00 μg/kg. A precision of ±30% provides intervals of confidence (95%) below the legal limit for animal feed for field contamination levels of ≤16.00 μg/kg. For a precision of ±10% a sample size of 6763 ears is required. A precision of ±10% provides intervals of confidence (95%) below the legal limit for animal feed for a field contamination level of ≤17.00 μg/kg.

As described before, it is possible to provide a single sample size for sample deviation of ±50, ±30, and ±10% about the true field concentration that can be used for every field contamination between 6.00 and 17.00 μg/kg. The above observation suggests that some simple guidelines may be used by farmers or any other stakeholder aimed at choosing a sampling plan for a specific field. Farmers and handlers can use eqs 1 and 2 or the results in **Tables 4** to choose the sample size needed for a level of precision that is considered to be acceptable to farmers and handlers.

Design of AFB_1 Sampling Plans for Ear Corn. From eq 2, the variability (precision) among sample test results can be estimated for various sample sizes and various field aflatoxin concentrations. Because of the variability among samples taken from the same field, the true AFB_1 concentration of a field of corn can never be determined with 100% certainty by measuring AFB_1 in samples taken from the field. As a result, there is a chance that a sample test result will indicate that a good field (i.e., a good field has a concentration below a regulatory limit) is bad (i.e., a bad field has a concentration greater than a regulatory limit) and there is a chance that a sample test result will indicate that a bad field is good. The chance of rejecting a good field is called the farmer's risk (false positive) and the chance of accepting a bad field is the processor's risk (false negative). The chances of accepting or rejecting fields with varying AFB_1 concentrations with a specific AFB_1 sampling plan design (sample size and accept/reject limit) can be estimated with an operating characteristic (OC) curve. As described previously(*15*, *16*) an OC curve can be constructed that describes the performances or risks of a specific aflatoxin sampling plan for ear corn knowing the variability among ear corn sample test results and the AFB_1 distributions among corn ears in the field.

The negative binomial (NB) distribution (*17*) was used to calculate OC curves for several sampling plan designs where NB parameters were calculated using variance eq 2. The effects of (a) increasing the sample size of a single sample, (b) increasing the

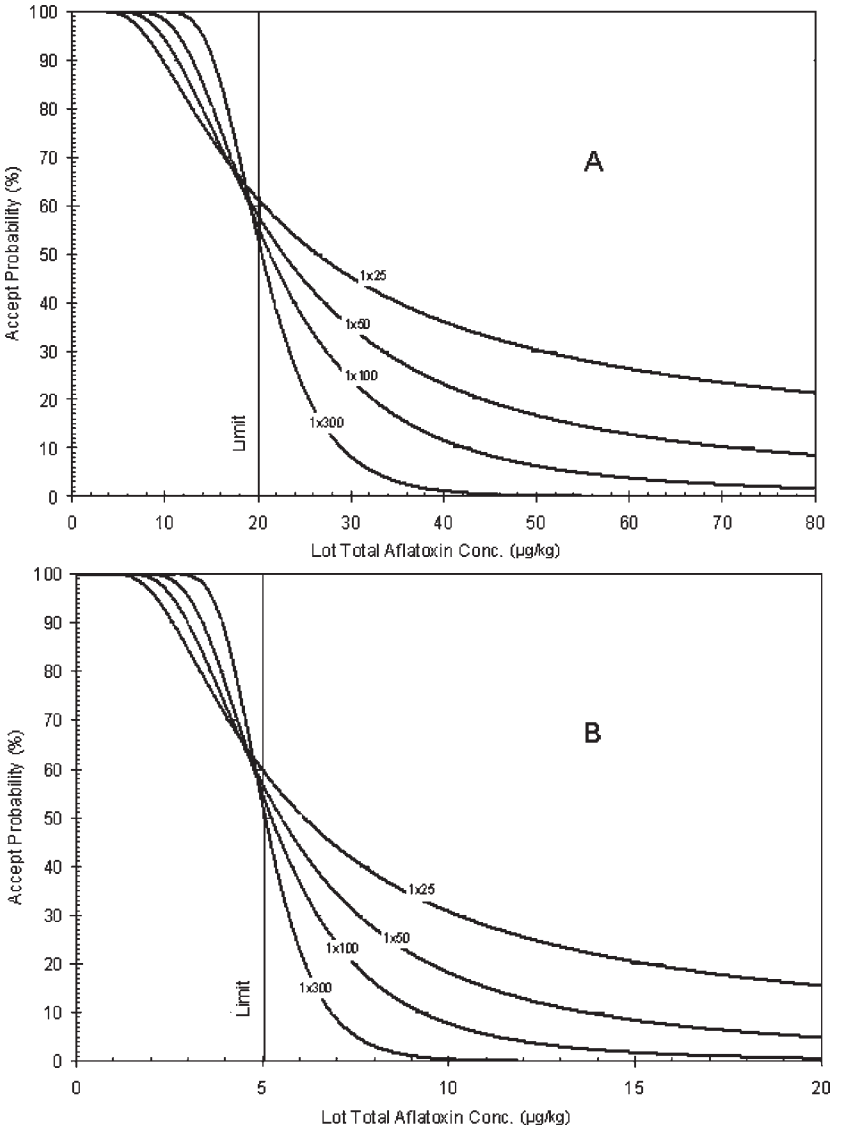

Figure 3. Operating characteristic curves showing the effect of increasing sample size (number of corn ears) on the chances of accepting and rejecting fields with various AFB_1 concentrations. All four sampling plans use an accept/reject limit equal to the regulatory limits of 20 μg/kg AFB_1 (**A**) and 5 μg/kg AFB_1 (**B**).

number of samples of a fixed size, and (c) decreasing the accept/reject limit relative to a regulatory limit on the probability of accepting and rejecting lots at various concentrations (OC curve) are described below. The OC curves demonstrate how to change a sampling plan design to minimize the chances of accepting bad fields (processor's risk) and rejecting good fields (farmer's risk). The effects that these three methods of altering sample design have on the probability of accepting and rejecting fields are described below for maximum or regulatory limits of 20 μg/kg for feed and 5 μg/kg for food.

Increasing Sample Size. Figure 3 shows the effect of increasing sample size from 25 to 50, 100, and 300 ears, corresponding to 5, 10, 20, and 60 kg, respectively, on the probability of accepting and rejecting fields at various AFB_1 concentrations when one attempts to determine if the field concentration is below the regulatory limits of 20 and 5 μg/kg, respectively, assuming about 200 g of shelled corn per ear of corn. In **Figure 3A**, the accept/reject limit for all four sampling plans is equal to the regulatory limit of 20 μg/kg. In **Figure 3B**, the accept/reject limit for all four sampling plans is equal to the regulatory limit of 5 μg/kg. With this type of sampling plan, all ears from a field are accepted if the sample test result is below the accept/reject limit; otherwise, the field is rejected (*11*).

Using **Figure 3**, one can see that the OC curve gets steeper, around the regulatory limit of either 20 or 5 μg/kg, as sample size increases. As the OC curve gets steeper, the probability of accepting good fields (field concentrations $\leq$ regulatory limit) increases and the probability of accepting bad fields (field concentrations $>$ regulatory limit) decreases. The probability of rejecting a field is equal to 100.0 minus the probability of accepting a field when expressed as a percent. Therefore, the probability of rejecting a good field (farmer's risk) and the probability of accepting a bad field (processor's risk) both decrease as sample size increases, regardless of the regulatory limit.

Several examples of how to read the OC curves are given below using **Figure 3A** for a 20 μg/kg regulatory limit. The probability of accepting all ears of corn from a field at 30 μg/kg (processor's risk) using a single sample of 25, 50, 100, or 300 corn ears is 46, 36, 26, and 9%, respectively. The probability of rejecting all corn ears from a field at 15 μg/kg (farmer's risk) is 26, 22, 19, and 9%, respectively. As shown in **Figure 3B**, when the regulatory limit is reduced to 5 μg/kg, the probability of accepting fields at high levels of aflatoxin are drastically reduced when compared to using an accept/reject limit of 20 μg/kg . For example, no fields above 10 μg/kg are accepted when using a sample of 300 ears and an

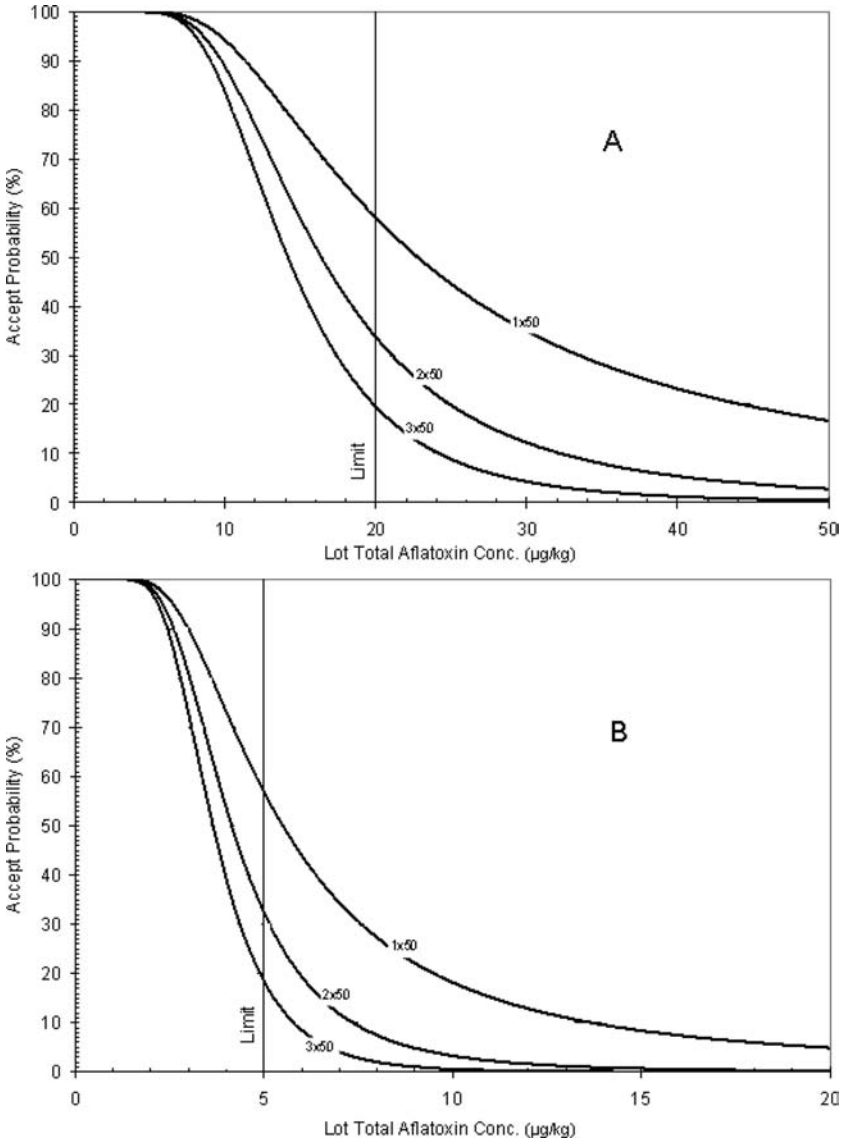

Figure 4. Operating characteristic curves showing the effect of increasing the number of 50 ear samples on the chances of accepting and rejecting fields with various AFB_1 concentrations. All three sampling plans use an accept/reject limit equal to the regulatory limits of 20 μg/kg AFB_1 (**A**) and 5 μg/kg AFB_1 (**B**).

accept/reject limit of 5 μg/kg. Increasing sample size has the positive result of decreasing both the farmer's and processor's risks at the same time. When choosing a sample size, one has to balance the cost (increase in sample size) versus benefits (reducing farmer's risk and/or processor's risk).

Increasing Number of Samples Tested or Attribute Type Design. **Figure 4** shows the effect of increasing the number of 50 ear (10 kg) samples tested for AFB_1 from 1 to 2 or 3 samples on the probability of accepting and rejecting fields with various AFB_1 concentrations in the attempt to determine if the field concentration is below the regulatory limits of 20 and 5 μg/kg. In **Figure 4A**, the accept/reject limit for all three sampling plans is equal to the regulatory limit of 20 μg/kg. In **Figure 4B**, the accept/reject limit for all three sampling plans is equal to the regulatory limit of 5 μg/kg. With this type of sampling plan, all ears from a field are accepted only if all samples test below the accept/reject limit, else the field is rejected (sample test results are not averaged). From **Figure 4**, one can see that the OC curves shift to the left as the number of samples (each sample size is 50 ears) increases. This attribute type design is similar in style to that currently used by the European Union for consumer-ready products such as peanuts.

Using **Figure 4A** as an example, the probabilities of accepting a field at 30 μg/kg using 1, 2, or 3 samples of 50 ears each are 35, 12, and 4%, respectively. The probability of accepting a bad field decreases as the number of samples tested increases. The effect of increasing the number of samples tested and requiring all samples to test $< 20\,\mu$g/kg to accept a field decreases the processor's risk. The probabilities of rejecting a lot at 15 μg/kg using 1, 2, or 3 samples of 50 ears each are 23, 41, and 55%, respectively. The probability of rejecting a good field increases as the number of samples tested increases. The effect of increasing the number of samples tested and requiring all samples to test $\leq 20\,\mu$g/kg to accept the field increases the farmer's risk. The OC curves in **Figure 4B** for a 5 μg/kg regulatory limit respond in a similar manner as the number of samples tested for aflatoxin increases. Increasing the number of samples tested and requiring all samples to test less than the accept/reject limit decreases the bad fields accepted (processor's risk) but increases the good fields rejected (farmer's risk). This type of design is best used late in the market system because it can be very costly to the farmer due to a large percentage of good fields that are rejected to obtain a low risk of accepting a bad field.

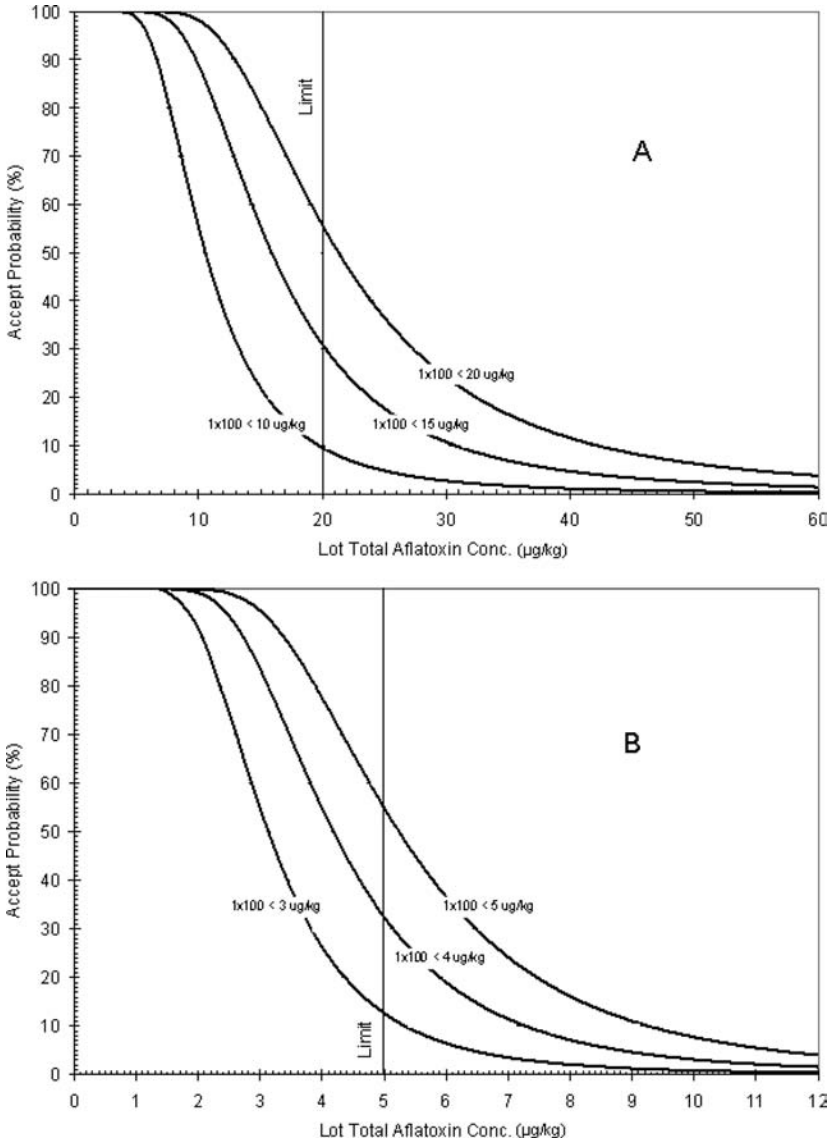

Figure 5. Operating characteristic curves showing the effect of decreasing the accept/reject limit relative to a regulatory limit of 20 μg/kg AFB_1 (**A**) or 5 μg/kg AFB_1 (**B**) for a given sample size (number of corn ears) on the chances of accepting and rejecting fields with various AFB_1 concentrations.

Decreasing the Accept/Reject Limit Relative to a Fixed Regulatory Limit. The effects of reducing the accept/reject limit relative to the regulatory limits of 20 and 5 μg/kg on the probability of accepting and rejecting corn fields is shown in **Figure 5**. **Figure 5A** shows the effect of decreasing the accept/reject limit from 20 to 15 or 10 μg/kg while the regulatory limit remains constant at 20 μg/kg on the probability on accepting and rejecting fields with various AFB_1 concentrations. **Figure 5B** shows the effect of decreasing the accept/reject limit from 5 to 4 or 3 μg/kg while the regulatory limit remains constant at 5 μg/kg on the probability on accepting and rejecting fields with various AFB_1 concentrations. In all cases, the sample size remains constant at 100 ears (20 kg). From **Figure 5**, the OC curves shift to the left as the accept/reject limit decreases below the regulatory limit of 20 or 5 μg/kg, respectively. Decreasing the accept/reject limit relative to a regulatory limit has a similar effect on the farmer's and processor's risk as described above for increasing the number of samples tested for AFB_1. Decreasing the accept/reject limit decreases the bad fields accepted (processor's risk) but increases the good fields rejected (farmer's risk). This type of design is also best used late in the market system because it can lower risk of accepting a bad field. Exporters often use this design to reduce the risk of consignments being rejected when they are retested at import.

Figure 5 is specific to a sample size of 100 ears. **Figure 6** also shows the effect of decreasing the accept/reject limit below regulatory limits of 20 and 5 μg/kg, but for a larger sample size of 300 ears (60 kg). **Figure 6** show how to use both the accept/reject limit and sample size to reduce the processor's risk, or the bad fields accepted, for two different regulatory limits of 20 and 5 μg/kg, respectively. Comparing OC curves with similar regulatory limits, the OC curves in **Figure 6A** are steeper due to the use of a larger sample than the corresponding curves in **Figure 5A** (20 μg/kg regulatory limit), and the OC curves in **Figure 6B** are steeper than the corresponding OC curves in **Figure 5B** (5 μg/kg regulatory limit).

When designing a mycotoxin sampling plan, one often specifies the desired risk levels (i.e., accept no more than 5% of fields at $\geq$25 μg/kg and reject no more that 5% of the fields at $\leq$15 μg/kg). Then, using the techniques of various sample sizes, numbers of samples, and/or accept/reject limits, the accept and reject probabilities can be computed (OC curve) for various sampling plan designs. If the costs of the sampling plan design are too big for the desired risk levels, then the final sampling plan design has to be

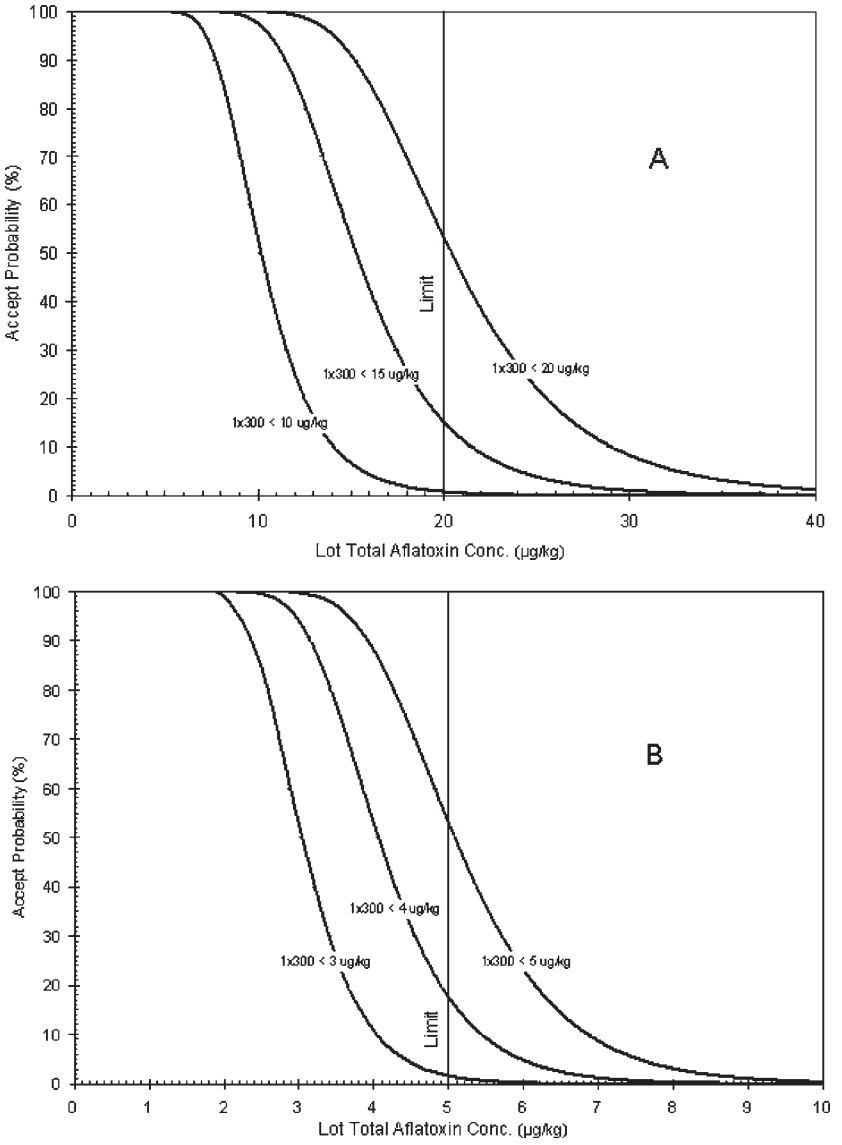

Figure 6. Operating characteristic curves showing the effect of decreasing the accept/reject limit relative to regulatory limits of 20 μg/kg AFB_1 (**A**) and 5 μg/kg AFB_1 (**B**) when using a sample of 300 ears on the chances of accepting and rejecting fields with various AFB_1 concentrations.

modified, which brings about a compromise between costs and levels of risk.

Sample Selection. All probability statistics described in this paper assume that samples are representative of the population from which the samples were taken, with no biases associated with the sample selection process. One method used to select a representative sample from a population is to combine many small incremental samples taken from many different locations in the population. It is more difficult to get a representative sample from a static population such as a field, trailer, or bin than to take incremental samples from a moving stream (dynamic population) as the corn ear is moved from one location to another.

Codex guidelines for sampling bulk commodities (*18*), such as peanuts and tree nuts, for aflatoxin recommend taking 100 incremental samples from a 20000 kg lot size, either static or dynamic. The recommended sampling rate is about one incremental sample per 200 kg of corn in the population. The recommended incremental sample size is 200 g, which is approximately the average mass of shelled corn on a single ear of corn. The 100 incremental samples are pooled to form an aggregate sample, which has to be equal to or larger than the required laboratory sample used to estimate the AFB_1 concentration of the corn in the field or the corn harvested from the field. If the aggregate sample is larger than required for the laboratory sample, a divider that provides random divisions is used to select the laboratory sample from the aggregate sample. For example, to select a 20 kg laboratory sample from a field, 100 incremental samples (assuming an ear of corn has 200 g of shelled corn) would have to be selected from the population.

The method used to select incremental samples is unique to the type of population (field, trailer, or conveyor) being sampled. The easiest sample selection method is probably associated with selecting incremental samples from a moving stream as the harvested corn is being moved from one location to another. Incremental samples are selected from the beginning to the end of the stream, usually at predefined time intervals depending on the flow rate of the stream. Sample selection methods for static populations such as a field are more complicated and require the field (all plants and all rows) to be divided into an $X-Y$ grid containing many cells. Then the incremental samples are taken in a random manner from some fraction of the total number of cells.

LITERATURE CITED

(1) Commission recommendation 2006/576/EC on the prevention and reduction of fusarium toxins in cereals and cereal products. *Off. J. Eur. Communities* **2006**, *L 234*/*35*.

(2) Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the european food safety authority and laying down procedures in matters of food safety. *Off. J. Eur. Communities* **2002**, *L 31/1*.
(3) Miraglia, M.; De Santis, B.; Minardi, V.; Debegnach, F.; Brera, C. The role of sampling in mycotoxin contamination: an holistic view. *Food Addit. Contam.: part A* **2005**, *22:1*, 31–36.
(4) ISO 2859-1. *Sampling Procedures for Inspection by Attributes*; International Organization for Standardization: Geneva, Switzerland, 1999.
(5) Brera, B.; Debegnach, F.; Minardi, V.; Pannunzi, E.; De Santis, B.; Miraglia, M. Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin B_1 in corn samples: interlaboratory study. *J. AOAC Int.* **2007**, *90*, 767–774.
(6) Durrett, R. In *Probability: Theory and Examples*, 2nd ed.; Duxbury Press: Pacific Grove, CA, 1995.
(7) Cochran, W. G. In *Sampling Techniques*, 3rd ed.; Wiley: New York, 1977.
(8) Bartlett, J. E., II; Kotrlik, J. W.; Higgins, C. Organizational research: determining appropriate sample size for survey research. *Inform Tech. Learn Perform J.* **2001**, *19*, 43–50.
(9) Robert, C. P.; Casella, G. In *Monte Carlo Statistical Methods*, 2nd ed.; Springer-Verlag: New York, 2004.
(10) Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* **2006**, *L 364/5*.
(11) Commission Directive 2003/100/EC amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. *Off. J. Eur. Communities* **2003**, *L 285/33*.
(12) SAS Institute Inc. In *SAS OnlineDoc 9.1.3*; SAS Institute: Cary, NC, 2002−2005.
(13) Fox, J. In *Applied Regression Analysis, Linear Models and Related Methods*; Sage Publications: Thousand Oaks, CA, 1997.
(14) Whitaker, T. B.; Dickens, J. W.; Monroe, R. J. Variability associated with testing corn for aflatoxin. *J. Am. Oil. Chem. Soc.* **1979**, *56*, 789–794.
(15) Whitaker, T. B.; Slate, A. B.; Jacobs, M.; Hurley, J. M.; Adams, J.; Giesbrecht, F. G. Sampling almonds for aflatoxin, part I: estimation of uncertainty associated with sampling, sample preparation, and analysis. *J. AOAC Int.* **2006**, *89*, 1027–1034.
(16) Whitaker, T. B.; Slate, A. B.; Hurley, J. M.; Giesbrecht, F. G. Sampling almonds for aflatoxin, part II: estimating risks associated with various sampling plan designs. *J. AOAC Int.* **2007**, *90*, 778–785.
(17) Evans, M.; Hastings, N.; Peacock, B. In *Statistical Distributions*, 3rd ed.; Wiley: New York, 2000.
(18) Codex Alimentarius Commission of the FAO/WHO. Proposed draft sampling plan for aflatoxin contamination in almond, brazil nuts, hazelnuts and pistachios, 38th session; The Hague, The Netherlands, **2006**.

Received for review January 26, 2010. Revised manuscript received June 11, 2010. Accepted June 12, 2010. The research project, named AFLARID, was financed by the Italian Ministry of Agriculture.