Food and Agriculture Organization
of the United Nations

Mycotoxin Sampling Tool

User Guide

Version 1.0 (December 2013)

Version 1.1 (updated September 2014)

Contents

Acknowledgements

Overview

Designing effective sampling plans for mycotoxin detection in food commodities is a complex task. This Mycotoxin Sampling Tool provides support in analyzing performance of sampling plans, and determining the most appropriate plan to meet user's defined objectives:

- The user can evaluate the effect of varying sampling plan design parameters, such as sample size, on the performance of the sampling plan.

- Using the performance information, the user can determine the most appropriate mycotoxin sampling plan to minimize risk of misclassifying lots considering available resources.

This User Guide provides step by step guidance on how to use the Mycotoxin Sampling Tool in 26 mycotoxin-commodity combinations.

Additional references on related topics can be found on the web at

http://www.bae.ncsu.edu/usda/www/whitaker1.htm

1.0 Introduction

Mycotoxins are toxic and/or carcinogenic metabolites produced by several fungi such as *Fusarium* and *Aspergillus* spp. Under certain environmental conditions, moulds that produce mycotoxins can grow on grains, nuts, and many other agricultural crops. The subsequent impacts of mycotoxin contamination of agricultural commodities on human and animal health as well as on national and international trade are increasingly concerning both developing and developed countries. A 2003 FAO/WHO survey indicated that about 100 countries regulate several mycotoxins in foods and feeds. It is important to be able to detect and quantify the mycotoxin concentration in foods and feeds destined for human and animal consumption and remove lots from the supply chain when the estimated lot concentration exceeds maximum levels established by food/feed regulatory agencies. The proper classification of commercial lots into acceptable and unacceptable categories can only be made correctly if the mycotoxin concentration in the lot can be estimated with a high degree of accuracy and precision.

1.1 Definition of a Sampling Plan

The mycotoxin concentration of a lot is usually estimated by measuring the mycotxin concentration in a small representative sample following a defined protocol called **mycotoxin sampling plan**. A mycotoxin sampling plan is defined by a **mycotoxin test procedure** and an **accept/reject limit**. For granular products such as treenuts, the mycotoxin test procedure consists of the three following steps:

- **<u>Sampling</u>** – The sampling step specifies how the sample will be taken from the bulk lot, the number of samples to be tested, and the size of each sample. It is assumed that the sample is selected in a random manner and is representative (no bias) of the lot. Typically many small incremental samples are selected from the lot and combined to form an aggregate sample. If the aggregate sample is larger than required, a laboratory sample is removed from the aggregate

sample and comminuted in a suitable mill. The smallest sample comminuted in mill is called laboratory sample.

- **Sample Preparation** – The sample preparation step is a two-part process where the sample is comminuted in a mill to reduce particle size and a small test portion is removed from the comminuted sample. It is assumed that the test portion is randomly selected from the comminuted sample.

- **Analytical step or Quantification** – In the analytical step, the mycotoxin is solvent extracted from the comminuted test portion and the mycotoxin concentration in an aliquot of the test portion/solvent blend is then quantified using approved analytical procedures.
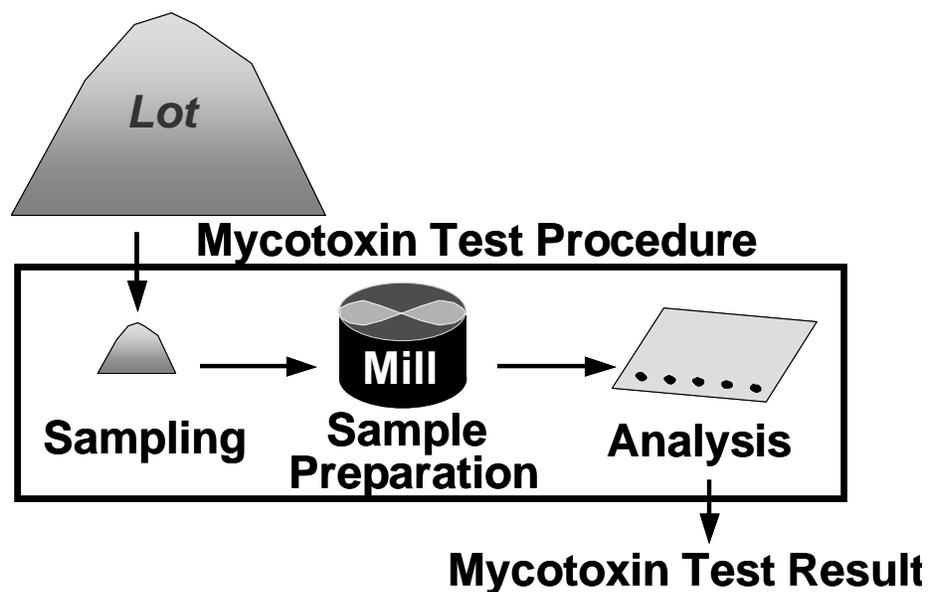
Figure 1. A mycotoxin-test procedure usually consists of a sampling, sample preparation and analytical steps.

The measured mycotoxin concentration in the test portion (called the sample test result) is (1) used to estimate the true mycotoxin concentration in the bulk lot or (2) compared to a defined accept/reject limit that is usually equal to a maximum level such as a regulatory limit. The accept/reject limit is a

threshold value, which may or may not equal a regulatory limit, and is used to classify lots into acceptable and unacceptable categories based upon the mycotoxin concentration in a sample(s) taken from the lot. With acceptance sampling the actual measured concentration is not as important as whether that concentration, and thus the lot concentration, is above or below a defined limit or maximum level.

1.2 Sampling Plan Evaluation

Because there is variability associated with each step of a mycotoxin test procedure (sampling, sample preparation and analysis), the true mycotoxin concentration in a bulk lot cannot be determined with 100% certainty. Differences between the laboratory sample concentration and the true lot concentration can lead to misclassification of lots. Two types of misclassifications are usually made when using a mycotoxin sampling plan to classify a lot based upon the lot's mycotoxin concentration. The first type of misclassification occurs when good lots (lots with mycotoxin levels below a defined maximum level) are rejected by the sampling plan (sample test result is greater than the accept/reject limit). This type of misclassification is called a false positive or the seller's risk. The second type of misclassification occurs when bad lots (lots with mycotoxin levels above a defined maximum level) are accepted by the sampling plan (sample test result is less than the accept/reject limit). This type of misclassification is called a false negative or the buyer's risk.

The frequency with which these two misclassifications occur depends upon the design of the sampling plan (number of laboratory samples, size of each laboratory sample, sample preparation method, analytical method, number of aliquots quantified, and accept/reject limit) and can be evaluated with the help of an operating characteristic (OC) curve. For a given mycotoxin sampling plan design, lots with a mycotoxin concentration C will be accepted with a certain probability P(A) and rejected with a certain

7

probability P(R). P(R) is equal to 1-P(A) when probability is expressed as a decimal. The acceptance

probability P(A) is equal to the probability that a sample test result, c, is less than or equal to an

accept/reject limit, C_a , for a given lot concentration C or P(A) = probability$(c \leq C_a \,|\, C)$. A plot of the

acceptance probabilities P(A) (expressed as a %) versus lot concentration C is called an OC curve

(**Figure 2**). The shape of an OC curve or the chances of accepting and rejecting lots is uniquely defined

by a given mycotoxin sampling plan design (mycotoxin test procedure and accept/reject limit).
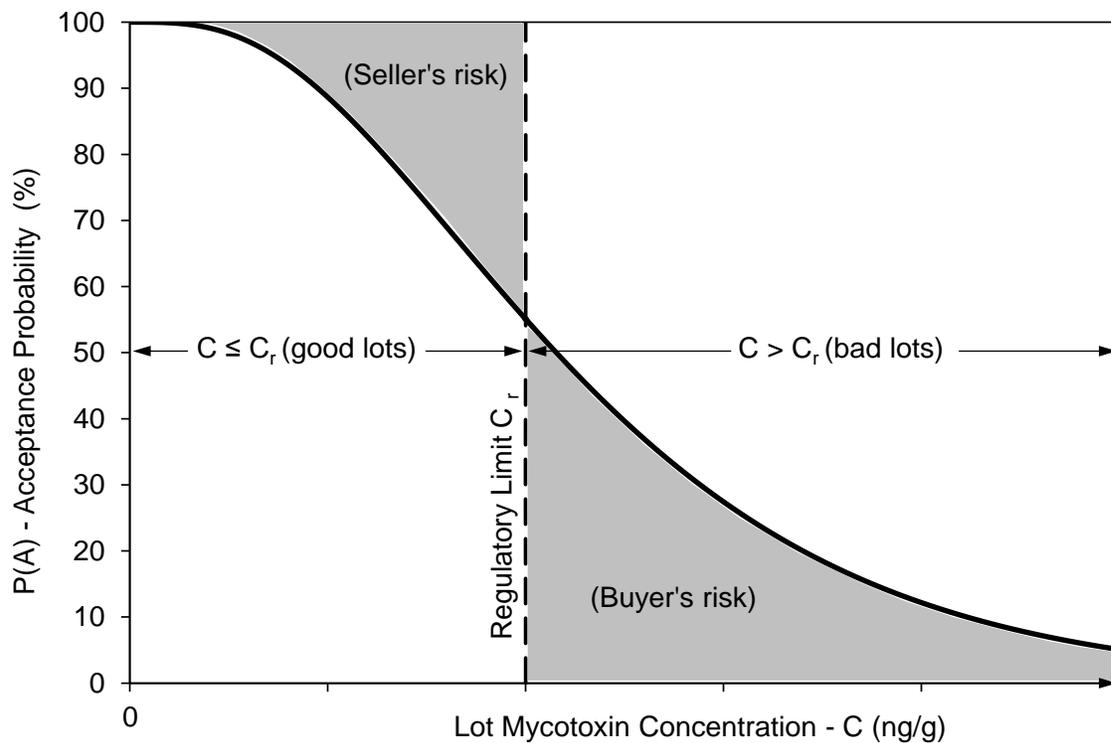
Figure 2. General shape of an operating characteristic (OC) curve. The shape of the OC curve is unique
for a mycotoxin test procedure and the accept/reject limit and indicates the magnitude of the buyer's
and seller's risks.

1.3 Procedures used to calculate accept probabilities or OC curve

Theoretical basis for the **Mycotoxin Sampling Tool** is provided in **Annex I**. Methods, based upon experimental measurements of the variability and distribution among replicated sample test results (taken from the same contaminated lot) along with the use of statistical theory, have been developed by researchers to calculate an operating characteristic (OC) curve that predicts the buyer's and seller's risks associated with a specific mycotoxin sampling plan design. To date, the variability and distribution for 26 different mycotoxin/commodity combinations (**Table A, Annex II**) have been studied and are described in publications cited in **Annex II**. Because it is difficult for the user to take the variance and distribution information and calculate OC curves for sampling plan designs of interest, this web based **Mycotoxin Sampling Tool** was developed so that users can simply input sampling plan design parameters (ie., sample size and accept/reject limits) and the **Mycotoxin Sampling Tool** will compute an OC curve and other information that will help the user to evaluate the performance of sampling plan designs and then choose a plan design that meet the user's objectives in terms of cost implications and risk levels.

2.0 How to Use the Mycotoxin Sampling Tool

The Mycotoxin Sampling Tool can be accessed at the following website address: http://www.fstools.org/mycotoxins/. The initial screen will appear with five Tabs: **Instructions, Edit Plans, Chart Results, Table Results, Plan Summary, and Export To Excel**. The function of each Tab is discussed below.

2.1 Instructions Tab

The user will find a brief overview of the Mycotoxin Sampling Tool along with links to the complete

User Guide and additional references.

2.2 Edit Plans Tab

In this Tab, the user describes the mycotoxin sampling plan design(s) to be evaluated by the

Mycotoxin Sampling Tool. Up to 10 different sampling plans can be simultaneously compared by the

Mycotoxin Sampling Tool. A copy of the screen in this **Edit Plans Tab** is shown below in **Table 1**

where the input boxes have been filled to describe a sampling plan to detect aflatoxin in shelled corn

(maize). The input screen in the **Edit Plans Tab** is laid out in three major sections: 1)

Mycotoxin/Commodity, 2) **Common Parameters**, and 3) **Plan Specific Parameters**. Each section is

described below.

Table 1. [Screen Shot – Edit Plans Tab] Input screen with input boxes for the **Mycotoxin Sampling Tool** showing the sampling plan design parameters needed to calculate an OC curve when sampling shelled corn (maize) for aflatoxin.

Mycotoxin Sampling Tool (Version 1.0)

| Instructions | Edit Plans | Chart Results | Table Results | Plan Summary | Export To Excel |

Edit Plans

Select a Mycotoxin and Commodity: Aflatoxin, Corn, Shelled

Common Parameters	**Allowable Range**	**Value**

Kernel Count Per kg:

This parameter refers to the number of units or number of kernels of the selected commodity in 1 kg. For the mycotoxin/commodity chosen, the suggested kernel count/kg or the number of kernels per unit mass is automatically shown. If your commodity of interest has a different kernel count/kg than shown in the **Kernel count per kg** input box, input a different kernel count/kg in the same box. Otherwise the suggested kernel count/kg will be used by default for the mycotoxin/commodity shown. There will be no kernel count/kg entry for the OTA/ginger combinations since the bulk lot for this product was in a powder form.

(1-100000)   3000

Regulatory Limit (ng/g):

The regulatory limit is a maximum level established by regulatory agencies, international organizations such as Codex, or industry groups. The regulatory limit defines the difference between good lots from bad lots. If exporting to several different countries, an exporter may have to design sampling plans for each importing country due to varying regulatory limits. This will require the use of different accept/reject limits as well.

(1-100)   15

Analytical Variance Type:

Research studies have shown that among lab analytical variability is larger than within lab analytical variability. When sampling plans are designed for an individual company or processor where a single lab is used to analyze a commodity for a mycotoxin, the "within lab" option should be selected. When multiple labs are used to analyze a commodity for a mycotoxin in an industry wide program, the "among lab" option should be selected. The among-lab analytical variance is approximately double the with-in lab analytical variance. Select the Analytical Variance Type to apply using the dropdown list.

Within Lab

Plan Specific Parameters

Plan Specific Parameters	Allowable Range	Plan 1 ✕	Plan 2 ✕	Add a Plan
Laboratory Sample Size - ns (kg): The laboratory sample is defined as the smallest size sample in kg taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box "**Add a Plan**". Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. The **Mycotoxin Sampling Tool** converts the sample size in kg to number of particles by multiplying the sample size in kg times the kernel count per kg.	(0.005-100)	5	5	
Number Laboratory Samples - scnt (#): The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results.	(1-300)	1	1	
Test Portion - nss (g): Each laboratory sample is comminuted in a suitable mill and a small test portion is removed from the comminuted laboratory sample. The mycotoxin is solvent extracted from the test portion and quantified using an approved analytical method.	(1-1100)	25	100	
Number of aliquots - na: An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used to quantify the mycotoxin concentration in the test portion. If more than one aliquot is specified, the tool assumes that all aliquot measurements are averaged in the quantification process.	(1-300)	1	1	
Accept/Reject Limit (ng/g): The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer.	(0-500)	15	15	

Save Plans

Select a Mycotoxin/Commodity: – The mycotoxin/commodity input box is a drop-down box that allows the user to choose one mycotoxin/commodity combination from a list of 26 combinations that are listed in **Table A, Annex II**. Place the cursor on the down arrow in the right corner of the mycotoxin/commodity box and a drop down list with 26 mycotoxin/commodity options will appear for selection. For this example, the combination of aflatoxin and shelled corn (maize) is chosen.

Select a Mycotoxin and Commodity: | Aflatoxin, Corn, Shelled | ▼ |

Common Parameters

The first three input parameters are called "**Common Parameters**" because they do not change for all sampling plans evaluated for the mycotoxin/commodity chosen. Parameter values inputted into the boxes must fall within the "**Allowable Range**" or an error message will occur. The three input boxes are:

- **Kernel Count per kg** – This parameter refers to the number of units or number of kernels of the selected commodity in 1 kg. For the mycotoxin/commodity chosen, the suggested kernel count/kg or the number of kernels per unit mass is automatically shown. If your commodity of interest has a different count/kg than shown in the **count per kg** input box, input a different count/kg in the same box. Otherwise the suggested count/kg will be used by default for the mycotoxin/commodity shown. There will be no count/kg entry for the OTA/ginger combinations since the bulk lot for this product was in a powder form. For the aflatoxin/shelled corn example in **Table 1**, the default count of 3000 kernels per kg was chosen for shelled corn (maize).

- **Regulatory Limit (ng/g)** - The regulatory limit is a maximum level established by regulatory agencies, international standards setting bodies such as Codex, or industry groups. The

regulatory limit defines the difference between good lots and bad lots. If exporting to several different countries, an exporter may have to design sampling plans for each importing country due to varying regulatory limits. This will require the use of different accept/reject limits as well. For the aflatoxin/shelled corn example, a maximum level or regulatory limit of 15 ng/g was chosen.

- **Analytical Variance Type** – Research studies have shown that among lab analytical variability is larger than within lab analytical variability. When sampling plans are designed for an individual company or processor where a single lab is used to analyze a commodity for a mycotoxin, the "within lab" option should be selected. When multiple labs are used to analyze a commodity for a mycotoxin in an industry wide program, the "among lab" option should be selected. There is a drop-down box where the user can choose one or the other. The **Mycotoxin Sampling Tool** assumes that the among-lab analytical variance is double the with-in lab analytical variance. For the aflatoxin/shelled corn example, analytical variability reflecting within lab variance was chosen.

Plan Specific Parameters

The next five parameters are plan specific and can be changed to determine the effect of various sampling plan design parameter(s) on the performance of up to ten sampling plans. For this example, we will compare the two aflatoxin sampling plans for shelled corn.

- **Laboratory Sample Size – ns (kg)** –The laboratory sample is defined as the smallest size sample in kg taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box "**Add a Plan**". Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. For Plans 1 and 2 a laboratory sample size of 5 kg has been entered. The **Mycotoxin**

Sampling Tool converts the sample size in kg to number of particles by multiplying the sample size in kg times the count per kg. For the aflatoxin/shelled corn example, sample size is 5 kg. The **Mycotoxin Sampling Tool** will use a laboratory sample size of 15,000 kernels (5 kg times 3000 kernels/kg) in the computation of the OC curve.

- **Number of Laboratory Samples – scnt (#)** – The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results. For example, the current Codex aflatoxin sampling plan for ready-to-eat treenuts requires two 10 kg samples both test less than a defined limit to accept the lot. For this example, 1 is entered into the input box for Plan 1 and Plan 2 to reflect that a single laboratory sample of 5 kg is selected from the lot for both sampling plans.

- **Test Portion Size – nss (g)** – Each laboratory sample is comminuted in a suitable mill and a small test portion is removed from the comminuted laboratory sample. The mycotoxin is solvent extracted from the test portion and its concentration is quantified using an approved analytical method. For the aflatoxin/shelled corn example, 25 g is entered into the Plan 1 box and 100 g is entered into Plan 2 box.

- **Number of Aliquots – na (#)** - An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used in the quantification of the mycotoxin concentration in the test portion. If more than one aliquot is specified, the **Mycotoxin Sampling Tool** assumes that all the aliquot measurements are averaged in the quantification process. For the aflatoxin/shelled corn example, 1 aliquot is entered into the box for Plan 1 and Plan 2.

- **Accept/Reject Limit (ng/g)** – The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer. Reasons for this requirement will be discussed later. For the aflatoxin/shelled corn example, an accept/reject limit of 15 ng/g is entered into each box for Plans 1 and 2.

There are two "Action Buttons", **Add a Plan** and **Save Plans**. They are described below:

- **Add a Plan** - Up to 10 different sampling plan designs can be entered in the **Plan Specific** boxes. Activate the "**Add a Plan**" button and a new field of five input parameter boxes will be displayed. Each sampling plan design entered into the boxes will be evaluated by the **Mycotoxin Sampling Tool**. Once all sampling plan designs have been entered, proceed to the "**Save Plans**" button. For this example, a second aflatoxin sampling plan design is specified where only the test portion size is changed from 25 to 100 g (all other parameters remain the same).

- **Save Plans** – This button should be activated once all sampling plans have been described. Two sampling plan designs described above in the Input Screen of the **Edit Plans Tab** will be evaluated. Both plans use a single laboratory sample of 5 kg to be selected from the lot using random sample selection techniques. The 5 kg laboratory sample (ns=5 kg) is comminuted (ground) in a Romer mill. In Plan 1, a 25 g test portion (nss=25 g) is removed from the comminuted laboratory sample. In Plan 2 a 100 g test portion (nss=100 g) is removed from the comminuted laboratory sample. The aflatoxin is quantified in 1 aliquot of corn/solvent blend (na=1 aliquot) using HPLC methods. For both plans, the sample test result will be compared to an accept/reject limit of 15 ng/g which is equal to the regulatory

limit of 15 ng/g. The lot is accepted if the sample test result is less than or equal to the accept/reject limit of 15 ng/g.

Once all of the mycotoxin sampling plan design parameters have been recorded in the Input Screen in the **Edit Plans Tab**, the **Mycotoxin Sampling Tool** will calculate accept probabilities and display them in graphical and tabular formats.

2.3 Chart Results Tab

Results for each sampling plan described in the input screen under the **Edit Plans Tab** are shown in **Chart Results Tab (Table 2)**. There are three sections in this tab: 1) chart controls, 2) variance information, and 3) OC curves.

Table 2. [Screen Shot – Chart Results Tab] Graphical results of acceptance probabilities for two sampling plans to detect aflatoxin in shelled corn.
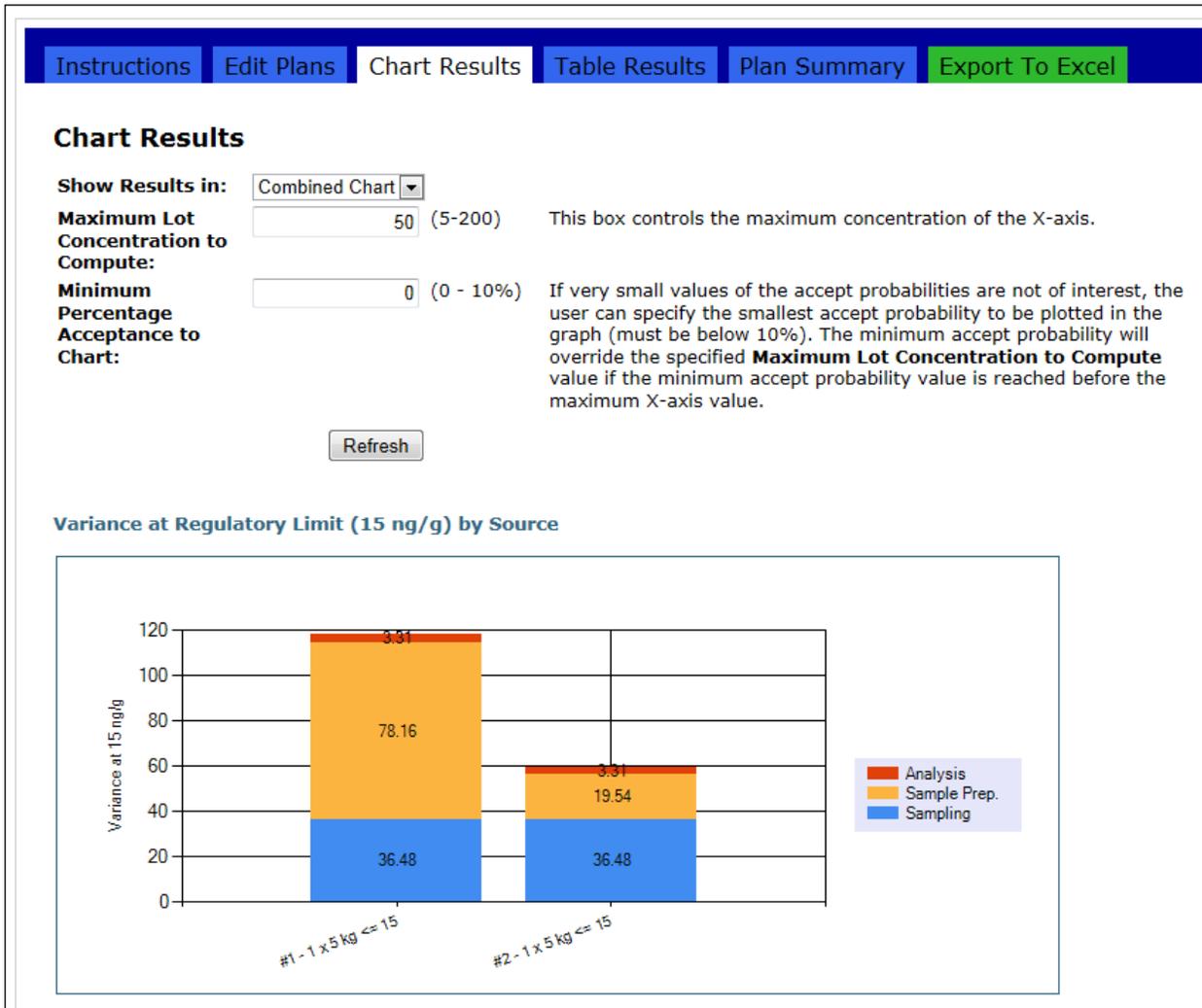
Percentage of Total Variance at Regulatory Limit (15 ng/g) by Source
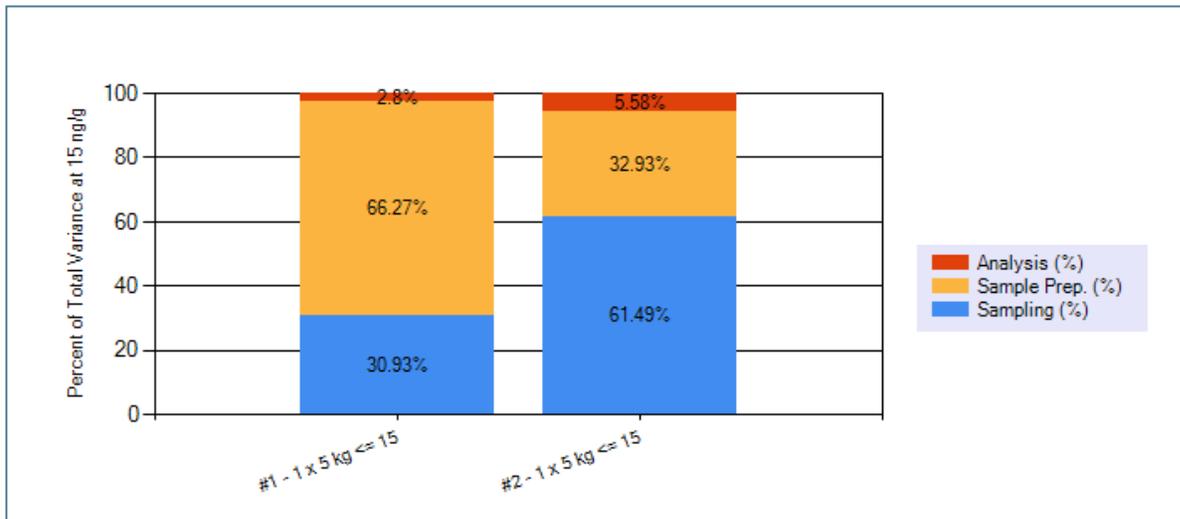
Probability of Accepting Lot of Corn, Shelled (%) for various lot concentrations using sampling plans described in the Edit Plans Tab. For a complete description about operating characteristic curves see Sections 1.2 and 2.3 in the User Guide.
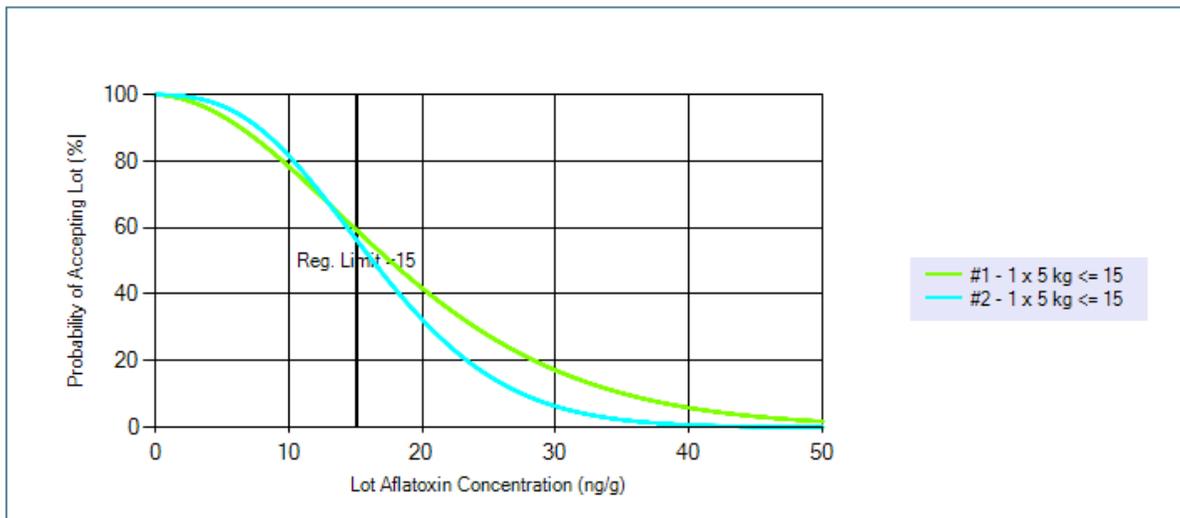
© FAO 2013 | Disclaimer | Acknowledgements

Chart Controls - At the top of Chart Results Tab (**Table 2)**, Chart Controls are provided so the user can control the graphical output of accept probabilities. The user has three ways to control the graph of

the OC curve(s): (1) **Show Results in:, (2) Maximum Lot Concentration to Compute:,** and **(3) Minimum Percentage Acceptance to Chart:.**

- **Show Results in**: - This drop-down box gives two choices, (1) **Combine Charts** or (2) **Separate Charts** . The user must choose one or the other style of graphical output for the OC curves. For the aflatoxin/shelled corn example, Combined Chart is chosen so the two OC curves for the two sampling plans that use different test portion sizes can be compared more easily.

- **Maximum Lot Concentration to Compute:** - This box controls the maximum concentration of the X-axis.

- **Minimum Percentage Acceptance to Chart:** - If very small values of the accept probabilities are not of interest, the user can specify the smallest accept probability to be plotted in the graph (must be below 10%). The minimum accept probability will over-ride the specified "**Maximum Lot Concentration to Compute**" value if the minimum accept probability value is reached before the maximum X-axis value.

Once all OC Graph Controls have been specified, the "**Refresh**" can be activated to plot the OC curve(s) as specified by the values in the **OC Graph Control** screen.

Variance Information – As part of the OC calculations, the sampling, sample preparation, analytical variances along with their total variance are also calculated (**Annex I, Equation I.1**). Since variances are a function of the mycotoxin concentration, the sampling, sample preparation, and analytical variances are calculated at the regulatory limit concentration C_r specified by the user in the **Edit Plans Tab** for the various sampling plan designs. The ratio of the sampling, sample preparation, and analytical variances to the total variance are also calculated at the regulatory limit concentration C_r specified by the user in the **Edit Plans Tab** for the various sampling plan designs. The variance values

and the variance ratios are shown as bar graphs in the **Chart Results Tab** (Table 2). The specific step

in the mycotoxin test procedure that has the highest ratio is a useful indication to the user which

sampling plan design parameter(s) (sample size, test portion size, and/or number of aliquots listed in

the Plan Specific Parameters) need to be adjusted to most effectively reduce variability of the

mycotoxin test procedure and thus reduce the number of lots misclassified by the sampling plan design.

For the aflatoxin sampling plan for shelled corn described in the input screen in the **Edit Plans Tab**,

the sampling, sample preparation, and analytical ratios are shown as bar charts in the **Chart Results**

Tab for each sampling plan.

OC Curves - For each sampling plan described in the input screen under the **Edit Plans Tab**, the

accept probabilities are calculated and plotted versus lot concentration. This plot is called an operating

characteristic or OC curve. The OC curves for the two sampling plans to detect aflatoxin in shelled

corn (maize) are shown in the **Chart Results Tab**. The probability of accepting a lot is expressed as a

percent (%) by multiplying the accept probability by 100. The OC curves show that the chances of

rejecting good lots and accepting bad lots is less when using a 100 g test portion when compared to

using a 25 g test portion.

2.4 Table Results Tab

The graphical information from the **Chart Results Tab** is shown in digital form in the **Table Results**

Tab (Table 3). The Table Results Tab shows Table Controls and digital values for Variance, Variance

Ratios, and OC curve probabilities.

Table 3. [Screen Shot – Table Results Tab] Tabular results of acceptance probabilities for two sampling plans to detect aflatoxin in shelled corn.

| Instructions | Edit Plans | Chart Results | Table Results | Plan Summary | Export To Excel |

Table Results

Maximum Lot Concentration to Compute:	40	(5-200)	Controls the last lot concentration value to show an accept probability in the table. Default values will be displayed automatically. The user can change the default value if desired.
Lot Concentration Increment:	5	(0.1-5)	Controls the incremental lot concentration value. A default value will be displayed automatically. The user can change the default value if desired.

Refresh

Variance at Regulatory Limit (15 ng/g) by Source

Source	#1 - 1 x 5 kg <= 15	#2 - 1 x 5 kg <= 15
Sampling	36.48	36.48
Sample Prep.	78.16	19.54
Analysis	3.31	3.31
Total	**117.95**	**59.33**

Percentage of Total Variance at Regulatory Limit (15 ng/g) by Source

Source	#1 - 1 x 5 kg <= 15	#2 - 1 x 5 kg <= 15
Sampling (%)	30.93	61.49
Sample Prep. (%)	66.27	32.93
Analysis (%)	2.8	5.58
Total (%)	**100**	**100**

Probability of Accepting Lot of Corn, Shelled (%) for various lot concentrations using sampling plans described in the Edit Plans Tab. For a complete description about operating characteristic curves see Sections 1.2 and 2.3 in the User Guide.

Lot Aflatoxin Concentration (ng/g)	#1 - 1 x 5 kg <= 15	#2 - 1 x 5 kg <= 15
0.000	100.0000	100.0000
5.000	93.6136	96.6531
10.000	78.4360	81.6868
15.000	59.6223	56.8322
20.000	41.8430	32.4391
25.000	27.5209	15.4956
30.000	17.1639	6.3438
35.000	10.2423	2.2742
40.000	5.8890	0.7268

© FAO 2013 | Disclaimer | Acknowledgements

Table Results control - For tabular output in **Table 3**, the user can control the length (maximum lot concentration) of the accept probability table(s) by inputting two parameters: (a) **Maximum Lot Concentration to Compute** and (b) **Lot Concentration Increment**.

- **Maximum Lot Concentration:** Controls the last lot concentration value to show an accept probability in the table. Default values will be displayed automatically. The user can change the default value if desired. For this example, a value of 40 ng/g is selected. The table of accept probabilities will stop at a lot concentration of 40 ng/g.

- **Lot Concentration Increment:** Controls the incremental lot concentration value. A default value will be displayed automatically. The user can change the default value if desired. For this example, a value of 5 ng/g is selected. The table of accept probabilities expressed as a % will display for lot concentrations in increments of 5 ng/g.

Once all Table Controls have been specified, the "**Refresh**" can be activated to generate the table(s) as specified.

Variances – In **Table 3**, the sampling, sample preparation and analytical variances are shown in table form for the two sampling plans described in the **Edit Plans Tab** (**Table 1**).

Variance Ratio - The bar charts of the variance ratios in **Chart Results Tab** are also digitized and are shown in the Table Results Tab (**Table 3)**.

OC Curve Accept Probabilities - It can be difficult to determine visually a precise value of the accept probability from the OC curve. Therefore, the accept probabilities, expressed as a percent (%), are also shown in an output table for each sampling plan design over a range of lot concentrations. The tabular output for the two OC curves in the **Chart Results Tab** is shown in the **Table Results Tab** (**Table 3)**. The table ends at a lot concentration of 40 ng/g and displays accept probabilities for lot concentrations in increments of 5 ng/g. Unlike **Table I.1** in **Annex I**, the **Table Results Tab** doesn't provide the reject probabilities, however, they are easily determined by subtracting the accept probabilities from 100%.

2.5 Plan Summary Tab

The **Plan Summary Tab** provides a quick overview of the sampling plan description specified by the user in the **Edit Plan Tab**. For this example, Plan #1 and Plan #2 for detecting aflatoxin in shelled corn (maize) are described in the **Plan Summary Tab** (**Table 4**).

Table 4. [Screen Shot – Plan Summary Tab] Summary Table describing sampling plan specified by the parameters used in the Edit Plans Tab.

2.6 Export To Excel Tab

For archival, presentations, and other uses, the information in each Tab can be transferred or copied to other software packages. For example, the accept probabilities **Table 3** in the **Table Results Tab** can be copied to a spreadsheet for further analysis and construction of plots.

Table 5. [Screen Shot – Export To Excel Tab] The Export To Excel Tab is used to export output shown in the Chart Results Tab and the Table Results Tab to an Excel spreadsheet.

3.0 Methods to reduce the buyer's and the seller's risks

The accept and reject probabilities or the shape of the OC curve is unique for a given sampling plan design (specific parameters such as ns, nss, na, and C_a for a given mill and analytical method). The accept and reject probabilities and thus the buyer's and seller's risks associated with a sampling plan design can be altered by changing one or more of the sampling plan design parameters (ns, nss, na, and C_a). The buyer's risk and seller's risk associated with a sampling plan design can be reduced by reducing the variability of the mycotoxin test procedure and/or lowering the accept/reject limit relative to the regulatory limit. The variability of the mycotoxin test procedure can be reduced by increasing laboratory sample size, the number of laboratory samples of a given size, increasing test portion size, number of test portions taken from the comminuted laboratory sample, and/or increasing the number of aliquots quantified by the analytical method. Reducing variability decreases both the buyer's and

seller's risks while reducing the accept/reject limit relative to the regulatory limit decreases the buyer's risk, but increases the seller's risk. Several examples using various mycotoxin/commodity combinations are given below to demonstrate how to alter the sampling plan design parameters to reduce the buyer's and/or seller's risks.

3.1 Increasing size of a single laboratory sample (ns)

The effect of increasing the size, ns, of a single laboratory sample from 5 to 10 to 20 kg on the accept and reject probabilities and the buyer's and seller's risks when sampling shelled peanut (groundnut) lots for aflatoxin was investigated with the **Mycotoxin Sampling Tool**. The input screen for the **Edit Plans Tab** is shown in **Table 6**. Using the button "**Add a Plan**", three sampling plan designs, each with a different laboratory sample size, were recorded in the input screen in the **Edit Plans Tab**. The values of test portion size (nss) and number of aliquots (na) remain constant at 250 g and 1 aliquot, respectively, for all three laboratory sample sizes. The accept/reject limit was set equal to the regulatory limit of 15 ng/g. The input screen is shown below in **Table 6.**

Table 6. [Screen Shot – Edit Plans Tab] Input screen in the Edit Plans Tab for three sampling plans using three different laboratory sample sizes to detect aflatoxin in shelled peanuts (groundnuts).

Plan Specific Parameters

	Allowable Range	Plan 1 ✕	Plan 2 ✕	Plan 3 ✕	Add a Plan
Laboratory Sample Size - ns (kg): The laboratory sample is defined as the smallest size sample in kg taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box **"Add a Plan"**. Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. The **Mycotoxin Sampling Tool** converts the sample size in kg to number of particles by multiplying the sample size in kg times the kernel count per kg.	(0.005-100)	5	10	20	
Number Laboratory Samples - scnt (#): The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results.	(1-300)	1	1	1	
Test Portion - nss (g): Each laboratory sample is comminuted in a suitable mill and a small test portion is removed from the comminuted laboratory sample. The mycotoxin is solvent extracted from the test portion and quantified using an approved analytical method.	(1-1100)	250	250	250	
Number of aliquots - na: An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used to quantify the mycotoxin concentration in the test portion. If more than one aliquot is specified, the tool assumes that all aliquot measurements are averaged in the quantification process.	(1-300)	1	1	1	
Accept/Reject Limit (ng/g): The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer.	(0-500)	15	15	15	

Save Plans

The performance of the three sampling plans designs is shown in graphical form in the **Chart Results Tab.** Using the **Combine Charts** option, the three OC curves, one for each sample size, are shown in **Figure 3** below.

Probability of Accepting Lot of Peanuts, Shelled (%) for various lot concentrations using sampling plans described in the Edit Plans Tab. For a complete description about operating characteristic curves see Sections 1.2 and 2.3 in the User Guide.
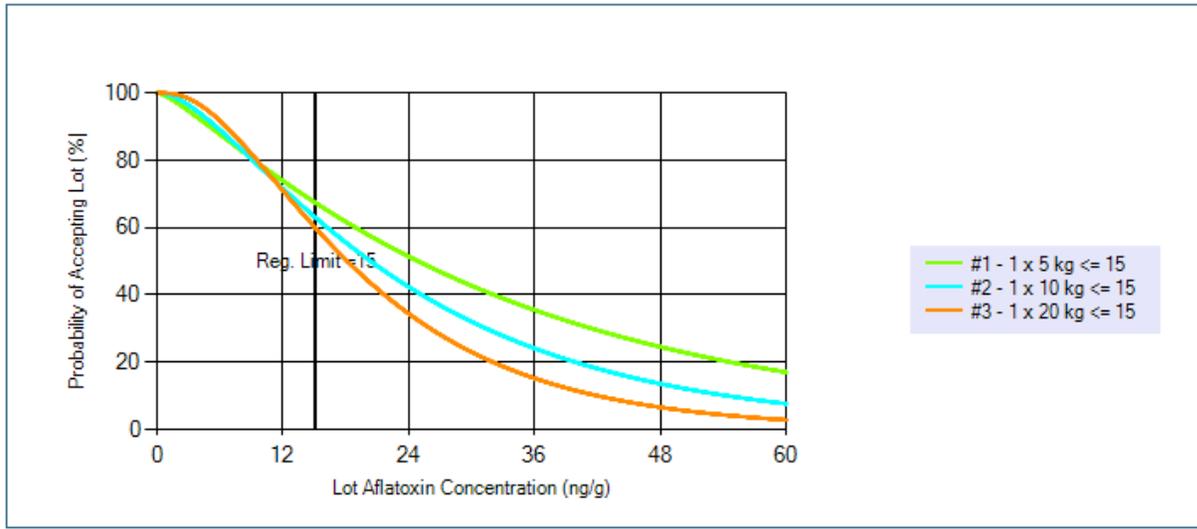
Figure 3. Three OC curves for sampling shelled peanuts (groundnuts) for aflatoxin using 5, 10, and 20 kg laboratory samples, USDA hammer mill, 250 g test portion, 1 aliquot, HPLC, and an accept/reject limit equal to the regulatory limit of 15 ng/g.

As the laboratory sample size increases from 5 to 10 to 20 kg, the slope of the OC curve (**Figure 3**) about the regulatory limit of 15 ng/g increases and the two areas associated with the buyer's and seller's risks (**Figure 2**) decrease. As a result, the areas representing the seller's risk and buyer's risk get smaller indicating that both risks get smaller as laboratory sample size increases. For example, as sample size increases from 5 to 10 to 20 kg, the chances of rejecting a lot at 5 ng/g (good lot) are 9.7, 7.9, and 5.0%, respectively. Also, as sample size increases from 5 to 10 to 20 kg, the chances of accepting a lot at 30 ng/g (bad lot) are 43.6, 33.1, and 24.3%, respectively. The accept probabilities can be seen in the **Table Results Tab** (not shown). A similar type of an effect (but to a lesser degree) can be obtained by increasing test portion size (nss) or number of aliquots (na) analyzed for aflatoxin. Increasing laboratory sample size is often the first approach taken to reduce uncertainty and risks because sampling accounts for most of the variability (89.8% at 15 ng/g for a 5 kg sample, (**Figure 4**) associated with the mycotoxin test procedure when using small laboratory sample sizes. The same

sample size effect has been also observed for other mycotoxin and commodities such as aflatoxins in

treenuts.
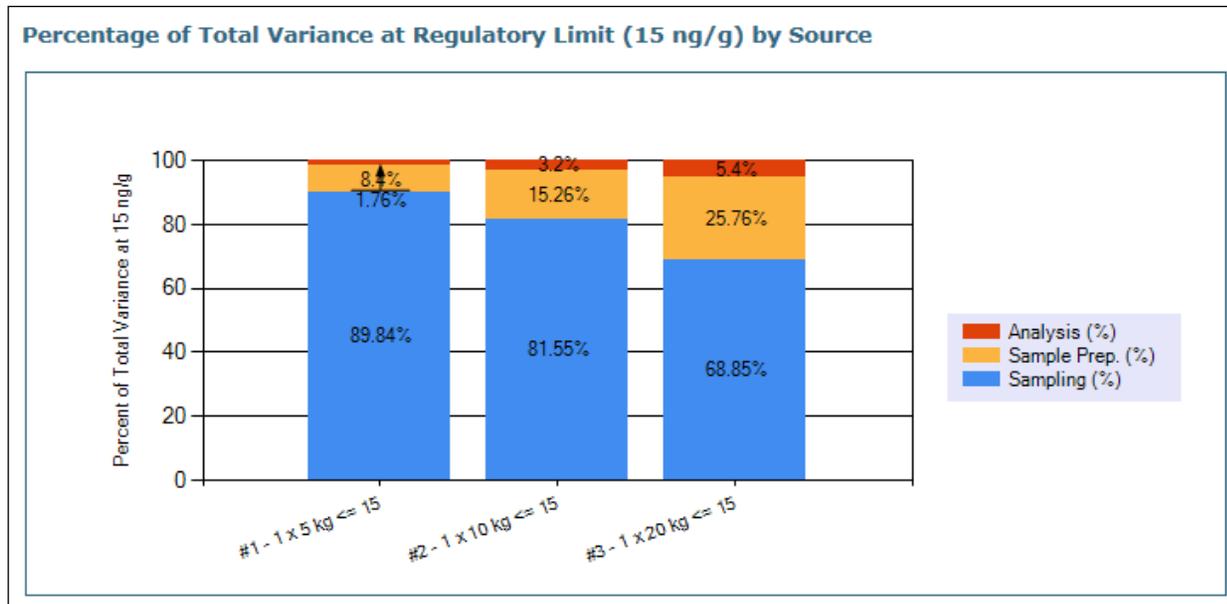
Figure 4. Percentage of the total variation that is contributed by the sampling, sample preparation, and analytical steps when sampling shelled peanuts (groundnuts) for aflatoxin at the regulatory limit of 15 ng/g aflatoxin.

As sample size increases, the sampling step becomes a smaller percentage of the total error of the

aflatoxin test procedure (**Figure 4**). However, the sample preparation step associated with the 250 g

test portion size becomes an increasingly significant percentage of the total error. At some point it no

longer makes sense to increase sample size above some level, but to increase the number or size of the

comminuted test portion analyzed for aflatoxin.

3.2 Increasing the number of laboratory samples

The effect of increasing the number of laboratory samples of a given size taken from a

contaminated lot on the accept and reject probabilities and on both the buyer's and seller's risks

when sampling shelled almond lots for aflatoxin was investigated with the **Mycotoxin**

Sampling Tool. If all sample test results are averaged, the effect is the same as increasing the size of a single laboratory sample (**Figure 3**). For example, the performance of a single 20 kg laboratory sample is approximately the same as measuring aflatoxin in two 10 kg laboratory samples and averaging the results. However, if all sample test results from multiple laboratory samples are required to test less than the accept/reject limit (no averaging), the effect on the OC curve and thus the buyer's and seller's risks are very different from averaging multiple sample test results.

The input screen for the **Edit Plans Tab** is shown in **Table 7**. Using the button "**Add a Plan**" in the Input Screen, the effect of selecting 1, 2, and 3x10 kg laboratory samples was evaluated with the **Mycotoxin Sampling Tool**. The values of a single laboratory sample (ns), test portion size (nss), and number of aliquots (na) remain constant at 10 kg, 100 g, and 1 aliquot, respectively, for all three sampling plans. The accept/reject limit was set equal to the regulatory limit of 10 ng/g. The input screen for the **Edit Plans Tab** is shown below in **Table 7**.

Table 7. [Screen Shot – Edit Plans Tab] Input screen for three sampling plans using 1, 2, or 3x10 kg samples to detect aflatoxin in shelled almonds.

| Instructions | Edit Plans | Chart Results | Table Results | Plan Summary | Export To Excel |

Edit Plans

Select a Mycotoxin and Commodity: Aflatoxin, Almonds, Shelled ▼

Common Parameters	**Allowable Range**	**Value**
Kernel Count Per kg:		
This parameter refers to the number of units or number of kernels of the selected commodity in 1 kg. For the mycotoxin/commodity chosen, the suggested kernel count/kg or the number of kernels per unit mass is automatically shown. If your commodity of interest has a different kernel count/kg than shown in the **Kernel count per kg** input box, input a different kernel count/kg in the same box. Otherwise the suggested kernel count/kg will be used by default for the mycotoxin/commodity shown. There will be no kernel count/kg entry for the OTA/ginger combinations since the bulk lot for this product was in a powder form.	(1-100000)	773
Regulatory Limit (ng/g):		
The regulatory limit is a maximum level established by regulatory agencies, international organizations such as Codex, or industry groups. The regulatory limit defines the difference between good lots from bad lots. If exporting to several different countries, an exporter may have to design sampling plans for each importing country due to varying regulatory limits. This will require the use of different accept/reject limits as well.	(1-100)	10
Analytical Variance Type:		
Research studies have shown that among lab analytical variability is larger than within lab analytical variability. When sampling plans are designed for an individual company or processor where a single lab is used to analyze a commodity for a mycotoxin, the "within lab" option should be selected. When multiple labs are used to analyze a commodity for a mycotoxin in an industry wide program, the "among lab" option should be selected. The among-lab analytical variance is approximately double the with-in lab analytical variance. Select the Analytical Variance Type to apply using the dropdown list.	Within Lab ▼	

Plan Specific Parameters	Allowable Range	Plan 1 ✗	Plan 2 ✗	Plan 3 ✗	Add a Plan
Laboratory Sample Size - ns (kg): The laboratory sample is defined as the smallest size sample in kg taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box "**Add a Plan**". Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. The **Mycotoxin Sampling Tool** converts the sample size in kg to number of particles by multiplying the sample size in kg times the kernel count per kg.	(0.005-100)	10	10	10	
Number Laboratory Samples - scnt (#): The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results.	(1-300)	1	2	3	
Test Portion - nss (g): Each laboratory sample is comminuted in a suitable mill and a small test portion is removed from the comminuted laboratory sample. The mycotoxin is solvent extracted from the test portion and quantified using an approved analytical method.	(1-1100)	100	100	100	
Number of aliquots - na: An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used to quantify the mycotoxin concentration in the test portion. If more than one aliquot is specified, the tool assumes that all aliquot measurements are averaged in the quantification process.	(1-300)	1	1	1	
Accept/Reject Limit (ng/g): The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer.	(0-500)	10	10	10	

Save Plans

The performance of the three sampling plans designs requiring either one, two, or three 10 kg laboratory samples (1x10kg, 10x2kg, and 3x10kg) to all test less than or equal to the accept/reject limit of 10 ng/g is shown in graphical form in the **Chart Results Tab**. Using the **Combine Charts** option, the three OC curves, one for each sample size, are shown in **Figure 5** below.
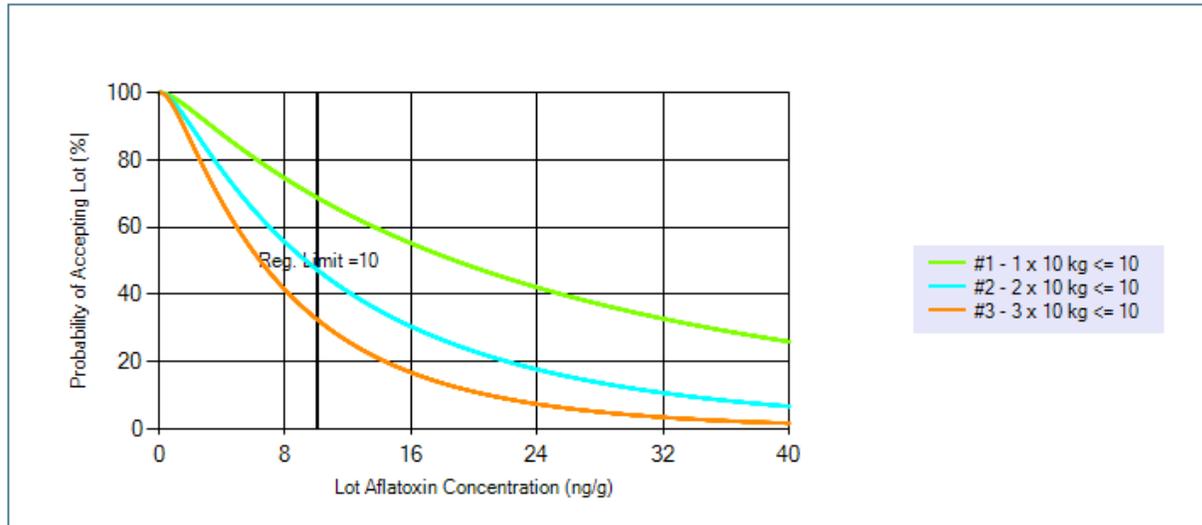
Figure 5. Three OC curves for sampling shelled almonds for aflatoxin using 1, 2, and 3 10 kg laboratory samples, USDA hammer mill, 100 g test portion, 1 aliquot, HPLC, and an accept/reject limit equal to the regulatory limit of 10 ng/g.

As the number of laboratory samples that are required to test less than or equal to the accept/reject limit increases, the OC curve shifts to the left reducing the buyer's risk (reduces the chances of accepting bad lots), but increasing the seller's risk (increases the chances of rejecting good lots). From the acceptance tables in the **Table Results Tab** (not shown), the chances of rejecting a lot at 5 ng/g (good lot) using 1x10kg, 2x 10kg, or 3x10kg samples are 15.3, 28.3, and 39.3%, respectively. Also, the chances of accepting a lot at 20 ng/g (bad lot) when using 1x10kg, 2x10kg, or 3x10kg samples are 49.1, 24.1, and 11.9%, respectively. This type of sampling plan is often used late in the marketing system for ready-to-eat products destined for human consumption in order to reduce the chances that a lot with a mycotoxin concentration greater than the regulatory limit will be accepted by the sampling plan. The buyer is placing most of the risk on the seller with this type of sampling plan design. An example of a sampling plan that uses multiple laboratory samples is that designed by Codex to detect

aflatoxin in ready-to-eat tree nuts. The Codex plan uses two 10 kg (2x10kg) laboratory samples where both laboratory samples must test less than the accept/reject limits of 10 ng/g total aflatoxin.

The bar graph shown in **Figure 6** indicates that the sampling step for this example is the largest contributor to the total error of the aflatoxin test procedure. The percentage of error is based upon one 10 kg laboratory sample since this is an attribute type sampling plan and there is no averaging of samples. The sampling step contributes a little over 93% of the total error.
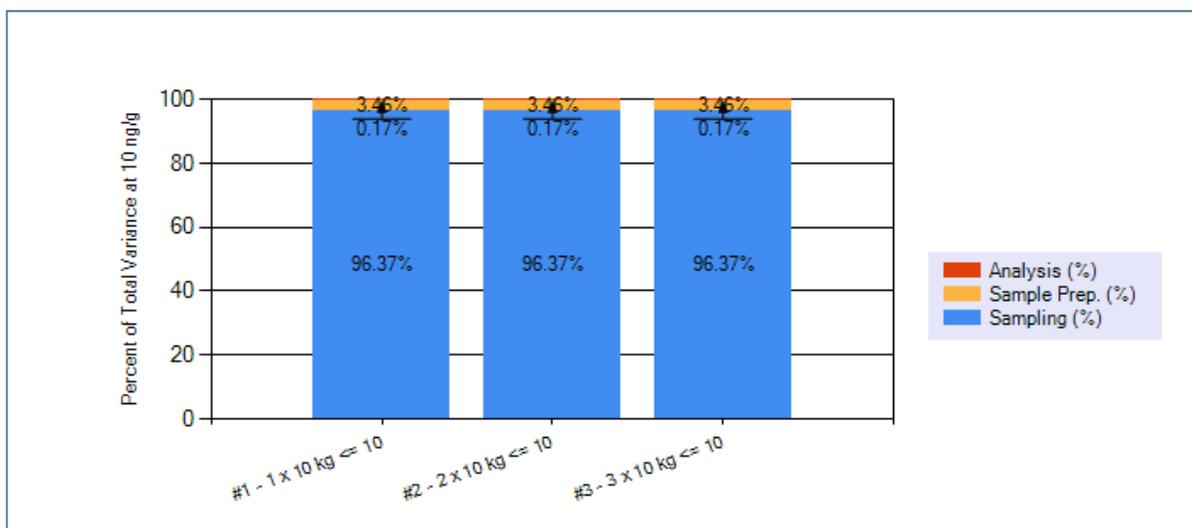
Figure 6. Percentage of the total variation that is contributed by the sampling, sample preparation, and analytical steps when sampling shelled almonds for aflatoxin using a 10 kg laboratory sample and an accept/reject limit equal to the regulatory limit of 10 ng/g aflatoxin.

3.3 Changing the accept/reject limit relative to the regulatory limit

The effect of changing the accept/reject limit relative to the regulatory limit on the buyer's risk and seller's risk when testing green coffee beans for ochratoxin A (OTA) was investigated using the **Mycotoxin Sampling Tool.** The input screen for the **Edit Plans Tab** is shown in **Table 8**. Using the

button "**Add a Plan**", three sampling plan designs where accept/reject limits of 15, 10, and 5 ng/g were

evaluated with the Mycotoxin Sampling Tool. The regulatory limit was held constant for all three

sampling plans at 15 ng/g. Sample size (ns), test portion size (nss), and number of aliquots (na) were

held constant at 1 kg, 100 g and 1 aliquot, respectively, for all three sampling plan designs.

Table 8. [Screen Shot – Edit Plans Tab] Input screen for three sampling plans using accept/reject limits of 15, 10, and 5 ng/g to detect ochratoxin A in green coffee beans.

Plan Specific Parameters	Allowable Range	Plan 1 ✕	Plan 2 ✕	Plan 3 ✕	Add a Plan
Laboratory Sample Size - ns (kg):					
The laboratory sample is defined as the smallest size sample in kg taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box "**Add a Plan**". Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. The **Mycotoxin Sampling Tool** converts the sample size in kg to number of particles by multiplying the sample size in kg times the kernel count per kg.	(0.005-100)	1	1	1	
Number Laboratory Samples - scnt (#):					
The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results.	(1-300)	1	1	1	
Test Portion - nss (g):					
Each laboratory sample is comminuted in a suitable mill and a small test portion is removed from the comminuted laboratory sample. The mycotoxin is solvent extracted from the test portion and quantified using an approved analytical method.	(1-1100)	100	100	100	
Number of aliquots - na:					
An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used to quantify the mycotoxin concentration in the test portion. If more than one aliquot is specified, the tool assumes that all aliquot measurements are averaged in the quantification process.	(1-300)	1	1	1	
Accept/Reject Limit (ng/g):					
The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer.	(0-500)	15	10	5	

Save Plans

The performance of the three sampling plans designs that use accept/reject limits of 15, 10, and 5 ng/g and a regulatory limit of 15 ng/g is shown in graphical form in the **Chart Results Tab**. Using the **Combine Charts** option, the three OC curves, one for each one for each accept/reject limit, are shown in **Figure 7** below.
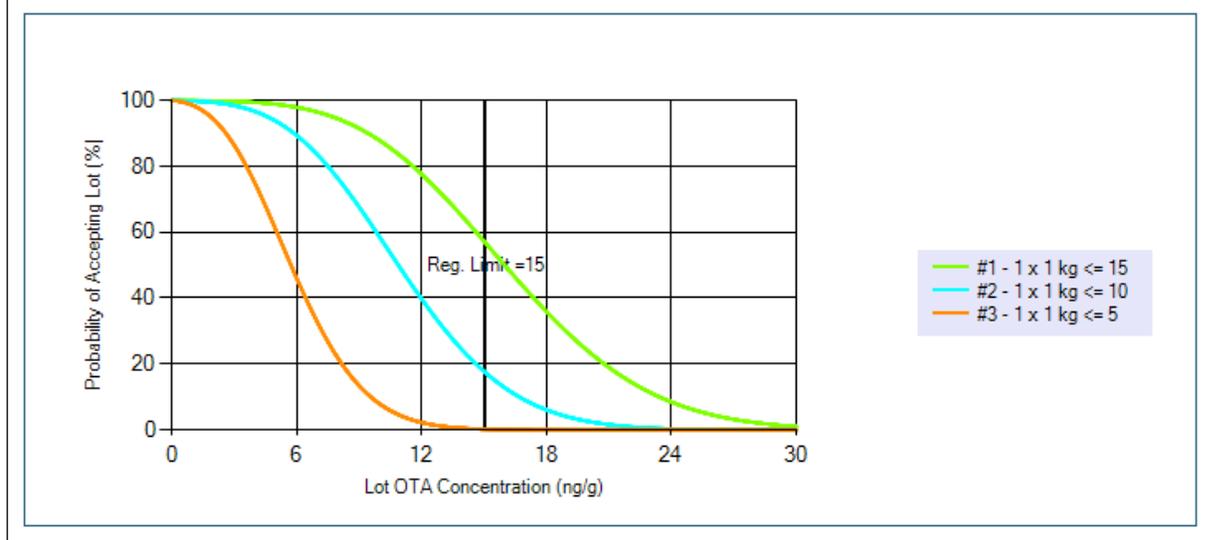
Figure 7. Three OC curves for sampling green coffee beans for ochratoxin A using accept/reject limits of 15, 10, and 5 ng/g with a regulatory limit of 15 ng/g. Sampling plan uses a single laboratory sample of 1 kg , Romer RAS mill, 100 g test portion, 1 aliquot, and HPLC.

Reducing the accept/reject limit to a value below the regulatory limit of 15 ng/g shifts the OC curve to the left which reduces the buyer's risk, but increases the seller's risk. From the acceptance tables in the **Table Results Tab** (not shown), the chances of rejecting a lot at 10 ng/g (good lot) using accept/reject limits of 15, 10, and 5 ng/g are 12.2, 41.6, and 92.1%, respectively. Also, the chances of accepting a lot at 20 ng/g (bad lot) when using accept/reject limits of 15, 10, and 5 ng/g are 23.9, 2.6, and 0.0%, respectively. Often, buyers prefer to contract with the seller for a product where the seller's sampling plan uses an accept/reject limit below the regulatory limit because it reduces the buyer's risk and forces the seller to take the largest share of the risk. Changing the accept/reject limit relative to the regulatory limit reduces only one of the two risks, but automatically increases the other risk.

The bar graph below in **Figure 8** indicates that the sampling step is the largest contributor to the total error of the aflatoxin test procedure for green coffee beans. The sampling step contributes a little over

79.9% of the total error.
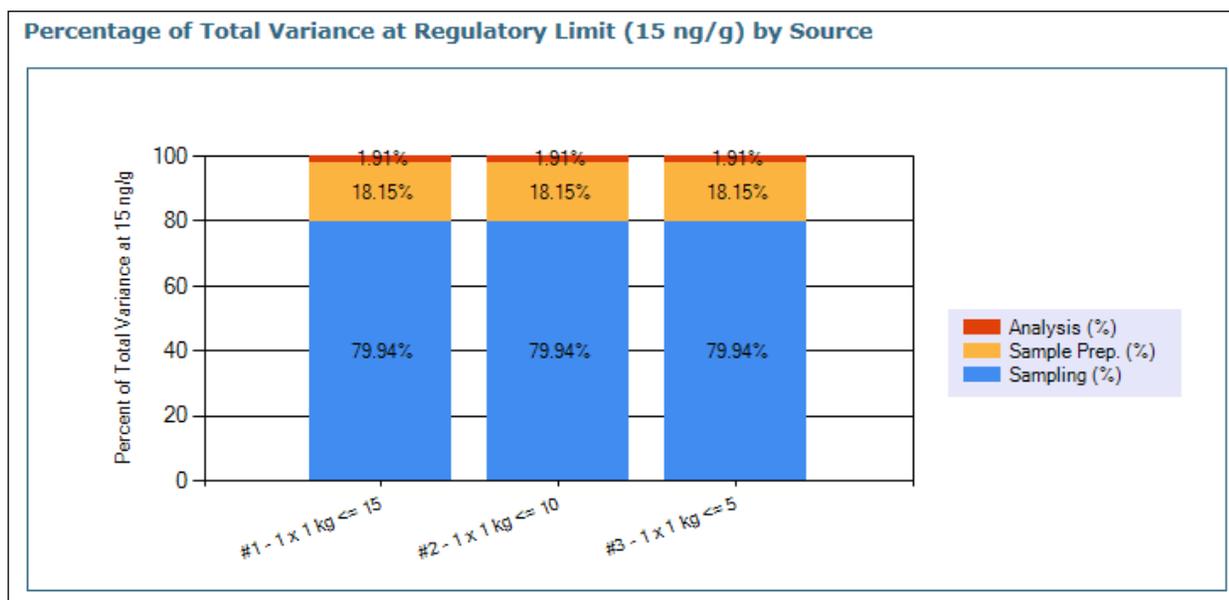
Figure 8. Percentage of the total variation that is contributed by the sampling, sample preparation, and analytical steps when sampling green coffee beans for ochratoxin A at the regulatory limit of 15 ng/g.

4.0 Limitation of Mycotoxin Sampling Tool

It is assumed that the sample is representative (no bias) of the lot from which the sample was taken. If the sample is not representative, additional uncertainty will be introduced into the sampling step of the mycotoxin test procedure that is not accounted for in the sampling variance shown in **Annex II**. Reference 8 in the Bibliography provides information on the proper methods to select representative samples from bulk lots.

The **Mycotoxin Sampling Tool** will not calculate OC curves for sequential type sampling plans because of the conditional probabilities associated with sequential plans. An example of a sequential type sampling plan is the aflatoxin sampling plan designed by the U.S. Department of Agriculture for shelled peanuts that uses either one, two, or three 21.8 kg (48 lbs) laboratory samples to make a decision to accept or rejects a lot for shipment to a food manufacturer. However, the basic probabilities

39

can be determined from the **Mycotoxin Sampling Tool** and used in other programming to calculate OC curves based upon conditional probabilities.

The **Mycotoxin Sampling Tool** will not compute OC curves for sampling plans that use dual limits. An example of this type plan is the EU aflatoxin sampling plan for ready-to-eat shelled almonds where two 10 kg laboratory samples must all test less than 8 ng/g AFB1 and 10 ng/g AFT to accept the lot. However, the OC curve for dual limits can be approximated by using a single accept/reject limit that is the average of the two limits. The OC curve for the EU sampling plan for shelled almonds can be approximated by using an accept/reject limit of 9 ng/g AFT in the Mycotoxin Sampling Tool.

The sample preparation variances in **Table A, Annex II** were measured for only one mill for each mycotoxin/commodity combination. In most cases, the mill chosen in the experiments produced a "dry-grind" for each mycotoxin/commodity. As a result, the estimated sample preparation variance associated with a mycotoxin test procedure will be slightly larger than if a "wet slurry grind" procedure is used in the sample preparation step. This provides, however, a small margin of safety in that predicted risks for dry grind will be higher than sample preparation using wet slurry grind (lower variability). The margin is probably small because the sample preparation variance is usually a small percentage of the total error when using small laboratory sample sizes.

Analytical variances used in the calculator for most mycotoxins/commodity combinations reflect the measured variability associated with HPLC. Analytical variances associated with other analytical methods, such as immunoassay methods and TLC have higher variances than HPLC. But the analytical variance is usually a small percentage of the total error and the use of immunoassay and TLC methods

instead of HPLC in the Mycotoxin Sampling Tool may not have a significant effect on the prediction of buyer's and seller's risks associated with a specific sampling plan design.

Sampling studies for a specific mycotoxin/commodity combination were usually conducted for a commodity growing in one geographical region. As a result, one will have to assume that the sampling statistics shown in **Table A, Annex II** will be the same even if the commodity is grown in other geographical regions. For example, the sampling statistics for almonds grown in California are assumed to be the same as almonds grown in Spain. Sampling statistics developed for fumonisin and shelled corn grown in south eastern USA were compared to sampling statistics for fumonisin and shelled corn grown in Nigeria, Africa and were found to be similar for both geographical regions.

Obviously, not all mycotoxin/commodity combinations of interest to users will be found in the core group of 26 mycotoxin/commodity combinations. If the user desires to calculate OC curves for a combination not in the core group of 26 combinations, they can choose a mycotoxin/commodity combination that may appear to be closely related to obtain an estimate of the performance of sampling plans for the desired combination not included in the core group of 26 combinations. For example, it may be appropriate to use sampling statistics for DON in wheat to estimate the performance of sampling plans for DON in oats.

5.0 Summary and Conclusion

It is important to be able to predict the buyer's and seller's risks associated with a sampling plan to detect mycotoxin in agricultural commodities. Once the magnitude of the buyer's and seller's risks are known, sampling plan design parameters, such as sample size, can be changed to make the risks more acceptable to the buyer and seller of products being traded. By changing sampling plan design

parameters, it is possible to adjust the performance of the sampling plan to meet user's objectives. Because of the computational complexities, a web-based Tool called a **Mycotoxin Sampling Tool** has been developed so users can design mycotoxin sampling plans for 26 different mycotoxin/commodity combinations. The Mycotoxin Sampling Tool is based upon experimental measurements of the variability and distribution among sample test results for these 26 mycotoxin/commodity combinations and the use of some basic statistical theory. The **Mycotoxin Sampling Tool** is programmed in such a way that additional mycotoxin/commodity combinations can be added to the web-based Tool once the sampling, sample preparation, and analytical variances have been determined.

6.0 Bibliography

1. Cullen, J.M. and Newberne, P.M. 1994. Acute Hepatotoxicity of aflatoxin. In: The Toxicology of Aflatoxin, eds: D.L.Eaton and J.D. Groopman, Academic Press, Inc. San Diego, CA, 921101, pp 3-26.

2. Council for Agricultural Science and Technology. 2003. Mycotoxins: Risks in Plant Animal, and Human Systems. Task Force Report Number 139. Ames. IA, 500014, pp. 199.

3. Food and Agriculture Organization (2003) *Worldwide Regulations for Mycotoxins in Food and Feeds in 2003.* FAO Food and Nutrition Paper 81. FAO, Viale della Terme di Caracalla, 00100, Rome, Italy. pp.165.

4. Codex Committee on Contaminants in Foods, 2008. Aflatoxin sampling plans for aflatoxin contamination in ready-to-eat treenuts and treenuts destined for further processing: almonds, hazelnuts, and pistachios, Alinorm 08/31/41, Appendix IX, 31st CAC Session, Geneva, Switzerland, 30 June to 4 July, 2008. www.**codex**alimentarius.net/download/report/700/al31_41e.pdf

5. Food and Agriculture Organization/World Health Organization. 2001. Proposed Draft Revised Sampling Plan for Total Aflatoxin in Peanuts Intended for Further Processing. Joint FAO/WHO Food Standards Program, CODEX Alimentarus Commission, 24th Session, Geneva, Switzerland, July 2-7, 2001. FAO/WHO Joint Office, Viale della Terme di Caracalla, 00100, Rome, Italy. pp.276-280.

6. Whitaker, T. B., Dickens, J. W., and Monroe, R. J. 1974. Variability of aflatoxin test results. J. Am. Oil Chem. Soc. 51:214-218.

7. Whitaker, T.B., Slate, A.B., Hurley, J.M., and Giesbrecht, F.G. 2006. Sampling almonds for aflatoxin, Part II: Estimating risk associated with various sampling plan designs. J. Assoc. Off. Anal. Chem., Int., 90:778-785.

8. Food and Agriculture Organization and International Atomic Energy Agency, 2010, *Sampling Procedures to Detect Mycotoxins in Agricultural Commodities*, Eds. Whitaker, T., Slate, A., Doko, B., Maestroni, B., and Cannavan, A. Springer, New York, NY, USA.

9. Whitaker, T. B., Horwitz, W., Albert, R., and Nesheim, S. 1996. Variability associated with methods used to measure aflatoxin in agricultural commodities. J. Assoc. Off. Anal Chem., Int., 79:476-485.

10. Horwitz, W., Albert, R., and Nesheim, S. 1993. Reliability of Mycotoxin Assays-An Update. J. Assoc. Off. Anal. Chem., Int., 76:461-491.

11. Horwitz, W. and Albert, R. 2006. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J. Assoc. Off. Anal. Chem., Int., 89:1095-1109.

12. Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. J. Royal Society of Chemistry, 125:385-386.

13. Whitaker, T.B., Slate, A.B., Jacobs, M., Hurley, J.M., Adams, J.G., and Giesbrecht, F.G. 2006. Sampling almonds for aflatoxin, Part I: Estimation of uncertainty associated with sampling, sample preparation, and analysis. J. Assoc. Off. Anal. Chem., Int., 89:1027-1034.

14. Shotwell, O. L., Goulden, M. L., Botast, R. J., and Hasseltine, C. W. 1975. Mycotoxins in hot spots in grains. 1. Aflatoxin and zearalenone occurrence in stored corn, Cereal Chem., 52:687.

15. Cucullu, A. F., Lee, L. S., Mayne, R. Y., and Goldblatt, L. A. 1986. Determination of aflatoxin in individual peanuts and peanut sections, J. Am. Oil Chem. Soc., 43:89.

16. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., and Young, J. H.. 2000. Testing shelled corn for aflatoxin, Part II: Modeling the distribution of aflatoxin test results. J. Assoc. Off. Anal. Chem., Int., 83:1270-1278.

17. Whitaker, T. B. and Dickens, J. W., Monroe, R. J., and Wiser, E. H. 1972. Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J. Am. Oil Chem. Soc. 49:590-593.

18. Whitaker, T. B. and Wiser, E. H. 1969. Theoretical investigations into the accuracy of sampling shelled peanuts for aflatoxin. J. Am. Oil Chem. Soc. 46:377-379

19. Giesbrecht, F. G. and Whitaker, T. B. 1998. Investigations of the problems of assessing aflatoxin levels in peanuts. Biometrics 54:739-753.

20. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., Franca, R.C.A. 2004. Testing green coffee for ochratoxin A, Part I: estimation of variance components, J. Assoc. Off. Anal. Chem., Int., 87:884-891.

21. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., and Franca, R.C.A. 2005. Testing green coffee for ochratoxin A, Part II: observed distribution of ochratoxin A test results, J. Assoc. Off. Anal. Chem., Int., 88:780-787.

22. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., and Franca, R.C.A. 2006. Testing green coffee for ochratoxin A, Part III: Performance of ochratoxin A sampling plan, J. Assoc. Off. Anal. Chem., Int., 89:1021-1026.

23. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., Young, J. H., and Bowman, D. T. 2000. Testing shelled corn for aflatoxin, Part I: Estimation of variance components. J. Assoc. Off. Anal. Chem., Int., 83:1264-1269.

24. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., Young, J. H. and Bowman, D. T. 2000. Testing shelled corn for aflatoxin, Part III: Evaluating the performance of aflatoxin sampling plans. J. Assoc. Off. Anal. Chem., Int., 83:1279-1284.

25. Whitaker T. B., A. B. Slate, J. G. Adams, T. Birmingham, F. G. Giesbrecht. 2010. Comparing the Performance of Sampling Plans that use a Single Regulatory Limit Based upon Total Aflatoxins to Sampling Plans that use Dual Limits Based upon B1 and Total Aflatoxins. World Mycotoxin Journal, 3 (1):35-44.

26. Whitaker, T. B. and Dickens, J. W. 1979. Evaluation of the Peanut Administrative Committee testing program for aflatoxin in shelled peanuts. Peanut Sci. 6:7-9.

27. Whitaker, T. B. and Dickens, J. W. 1989. Simulating aflatoxin testing plans for shelled peanuts in the export market. J. Assoc. Off. Analytical Chem. 72:644-648.

28. Whitaker, T. B., Trucksess, M., Johansson, A. S., Giesbrecht, F. G., Hagler, W. M., Jr., Bowman, D. T. 1998. Variability associated with testing corn for fumonisin. J. Assoc. Off. Anal. Chem., Int., 81:1162-1168.

29. Whitaker, T.B., Doko, M. B., Maestroni, B. M., Slate, A. B., and Ogunbanwo, B. F. 2007. Evaluating the performance of sampling plans to detect fumonisin B_1 in maize lots marketed in Nigeria. J. Assoc. Off. Anal. Chem., Int., 90:1050-1059.

7.0 ANNEX I - Theoretical Basis for Mycotoxin Sampling Tool

7.1 Mycotoxin sampling studies

Methods, based upon experimental measurements of the variability and distribution among replicated sample test results (taken from the same contaminated lot) along with the use of statistical theory, have been developed by researchers to calculate an operating characteristic (OC) curve that predict the buyer's and seller's risks associated with a specific mycotoxin sampling plan design. These experimental results have been incorporated into the **Mycotoxin Sampling Tool** to calculate the performance (OC curves) of mycotoxin sampling plans. To date, the variability and distribution for 24 different mycotoxin/commodity combinations (**Table A, Annex II**) have been studied and are described in publications cited in **Annex II**. Sixteen of the 26 sampling studies are related to aflatoxins, either total (AFT) or B1 (AFB1), while the commodities vary from peanuts to powdered ginger. Aflatoxin is the prevalent mycotoxin studied because regulatory agencies considered aflatoxin to be the most serious food contaminant of the various known mycotoxins. Other mycotoxins studied include fumonisin (F), ochratoxin A (OTA), and deoxynivalenol (DON). References are cited in **Table A, Annex II** that describes each of the 26 sampling studies (manuscripts that describe aflatoxin sampling studies for dried figs are still in progress). A link to many of these publications can be found in the **Instructions Tab**. Basic objectives of each sampling study shown in **Table A, Annex II** were (a) to measure the sampling, sample preparation, and analytical variability associated with testing a specific commodity for a specific mycotoxin, (b) to measure the mycotoxin distribution among replicated sample test results taken from the same contaminated lot, and (c) demonstrate how to use the variance and distribution information to calculate the performance (OC curve or seller's risk and buyer's risk) for various sampling plan designs.

7.2 Variability estimates among sample test results

The total variability associated with a mycotoxin test procedure is the sum of the sampling variability, sample preparation variability, and analytical variability (**Figure I.1**). The term "Error" used in **Figure I.1** indicates random error of variability.
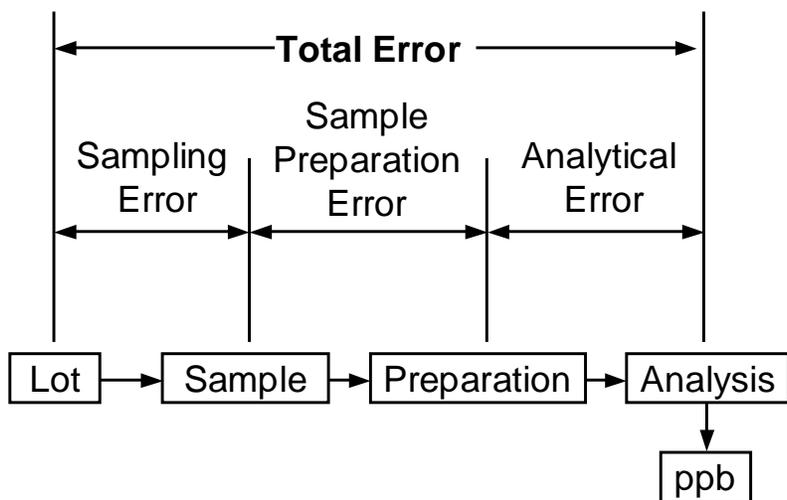
Figure I.1. Uncertainty associated with a mycotoxin test procedure.

In all studies, variance was used as the basic measure of variability because variances are additive (unlike the standard deviation). As shown in **Equation I.1,** the total variance (s^2_t) associated with a mycotoxin test procedure is the sum of the sampling variance (s^2_s), sample preparation variance (s^2_{sp}), and analytical variance (s^2_a). The theoretical assumptions associated with the use of **Equation I.1** are described by Whitaker et al., 1972 (**Ref 6, Bibliography**).

$$s^2_t = s^2_s + s^2_{sp} + s^2_a \hspace{3cm} \text{(I.1)}$$

The sampling, sample preparation, and analytical variances were measured experimentally for one laboratory sample size (ns), one test portion size (nss) when the laboratory sample is comminuted in a specific mill, and when the mycotoxin is quantified in one aliquot (na) taken from the test portion/solvent blend solution as specified by a specific analytical method.

A basic assumption underlying all variance measurements is that no biases were introduced in the selection of the sample from the lot, or the selection of the test portion from the comminuted laboratory sample, or the quantification of the mycotoxin in the test portion. A detailed description of how to select a representative (no bias) sample from a bulk lot is described in a joint publication by FAO and IAEA, 2010 (**Ref 8, Bibliography**). The analytical measurements associated with each mycotoxin/commodity combination were conducted in a single laboratory. Therefore, the analytical variability in each of the 26 sampling studies reflects within-lab variance. A study by Whitaker et al., 1996 (**Ref 9, Bibliography**) using an analytical database developed by Horwitz et al., 1993 (**Ref 10, Bibliography**), determined that the among-lab analytical variance was approximately twice that of the within-lab variability regardless of type analytical method (HPLC, TLC, etc.). Other generalized estimated of among-lab analytical variance were determined by Horwitz et al., 2006 (**Ref 11, Bibliography**) and Thompson, 2000 (**Ref 12, Bibliography**). Among-lab analytical variance estimates are shown in **Studies 27, 28, and 29 in Table A, ANNEX II**. The **Mycotoxin Sampling Tool** will use by default the with-in lab estimates of analytical variance shown in **Table A, ANNEX II**. However, the **Mycotoxin Sampling Tool** allows the user to specify among-lab analytical variance by doubling the with-in lab variance when calculating an OC curve for a specific sampling plan design for a specific mycotoxin/commodity combination.

It was cost effective to measure, for example, the sampling variance for only one laboratory sample size because statistical theory states that if you double sample size, the variance is reduced by half. Using this theory, researchers can save on resources and use statistical theory to predict the sampling variance for any other laboratory sample size (ns) in number of particles based upon the measured variance at one sample size. The same approach was used to measure the sample preparation variance for any test portion size (nss) in grams and the analytical variance for any number of aliquots (na) quantified by the analytical procedure. The one exception to the above approach was sampling studies to detect aflatoxin in farmer's stock (inshell) peanuts where sampling variance was determined for three sample sizes (**Ref 7, 8, and 9, Annex II**). In that study, sampling variance decreased as sample size increased in an amount predicted by statistical theory. Following the advice from statistical support, studies were designed to maximize the number of replicated samples, test portions, and aliquots tested for a mycotoxin along with the number of lots tested for a given level of resources instead of using resources to determine the effect of sample size on variability.

The sampling, sample preparation, and analytical variances were measured using multiple lots with varying mycotoxin concentration levels C since studies showed that all three of the above variance components (sampling, sample preparation, and analytical) are a function of the mycotoxin concentration C (**Ref 13, Bibliography**). The number of lots studied varied from study to study depending on resources, but an effort was made to use at least 20 lots where each lot had a different mycotoxin level. Each lot provided one estimate of sampling, sample preparation, and analytical variances and mean mycotoxin concentration. Then, regression equations were developed for each variance component as a function of the mycotoxin concentration C. The sampling, sample preparation, and analytical variance equations from the regression analysis are shown in **Table A, Annex II** for each mycotoxin/commodity combination studied. The sampling, sample preparation, and analytical

variance equations in **Table A**, **Annex II** have been generalized so that the sampling, sample preparation, and analytical variance can be calculated for any given sample size (ns), test portion size (nss), and any number of aliquots (na) quantified.

The total variance associated with a mycotoxin test procedure is not shown in **Table A, Annex II**, but can be determined by summing (**Equation I.1**) the sampling, sample preparation, and analytical variance equations shown in **Table A, Annex II**. For example, the total variance associated with testing shelled corn (maize) for aflatoxin using a sample of ns kernels, Romer mill, a test portion of nss grams, and quantifying aflatoxin in na aliquots using HPLC is

$$s^2_t = (3390/ns)11.36C^{0.98} + (50/nss)1.254C^{1.27} + (1/na)0.143C^{1.16} \qquad (I.2)$$

For an aflatoxin test procedure that uses a 1 kg (3000 kernels) laboratory sample, 50 g test portion taken from the laboratory sample that was comminuted in a Romer RAS mill, and quantifying aflatoxin in one aliquot using HPLC (ns= 3000, nss = 50, and na=1) to estimate aflatoxin in a bulk lot of shelled corn (maize) at 20 ng/g (C=20 ng/g), the sampling, sample preparation, analytical, and total variances are: $s^2_s = 241.8$; $s^2_{sp} = 56.3$; $s^2_a = 4.6$; and $s^2_t = 302.7$, respectively (**Equation I.2**). The standard deviation (SD) and the coefficient of variation (CV) for each step of the mycotoxin test procedure can be calculated from the above variances if desired. Typically the variance and standard deviation increase with concentration, but the coefficient of variation (also called the relative standard deviation) decreases with an increase in concentration.

For the above example, sampling, sample preparation, and analytical steps of the aflatoxin test procedure account for 79.9 (241.8/302.7), 18.6 (56.3/302.7), and 1.5% (4.6/302.7) of the total

variability of the aflatoxin test procedure example described above to measure aflatoxin in shelled corn (maize), respectively. When designing a mycotoxin sampling plan it is important to know how much each step of the mycotoxin test procedure contributes to the total variability so that resources can best be utilized to reduce the total variability of the mycotoxin test procedure. In the above example, best use of resources may be to increase the size of the laboratory sample since it accounts for almost 80% of the total variability. The variance equations shown in **Table A, Annex II** are also used as distribution parameters to calculate the distribution of sample test results associated with a specific sampling plan design used to test a specific commodity for a specific mycotoxin.

7.3 Mycotoxin distribution among sample test results

The large sample to sample variability for granular products (ie., treenuts) is due in part to the extreme mycotoxin distribution among individual particles in a bulk lot. Research indicates that a very small percentage (less than 1%) of particles in a bulk lot is contaminated and that the mycotoxin concentration on individual contaminated particles can vary from low levels to extremely high levels. For example, Shotwell (**Ref 14, Bibliography**) and Cucullu (**Ref 15, Bibliography**) observed aflatoxin levels as high as 4×10^5, 1×10^6, and 5×10^6 ng/g on a single corn kernel, a single peanut kernel, and a single cotton seed, respectively.

To calculate an OC curve, it is important to be able to describe the mycotoxin distribution among individual particles in a bulk lot. However, it is too expensive and time consuming to measure the mycotoxin concentration among individual particles in a lot because it would take a very large number of particles to construct the mycotoxin distribution among individual particles for a given lot concentration. If only one particle per 1000 particles is contaminated, one would have to measure hundreds of thousands of particles to describe the particle to particle mycotoxin distribution. Most of

the particles would test less than the limit of detection of the analytical method. As a result, researchers have taken the approach to select multiple samples of a given size or number of particles from a contaminated lot, quantify the mycotoxin in each sample, construct an observed mycotoxin distribution among the multiple sample test results (from the same lot), and using goodness of fit methods find a theoretical distribution to simulate the observed mycotoxin distribution among sample test results (**Ref 16 and 17, Bibliography**). Then the measured mycotoxin distribution among sample test results is used to predict statistically the mycotoxin distribution among individual particles in a lot.

Sampling studies (**Ref 17 and 18, Bibliography**) concerned with the detection of aflatoxin in shelled peanuts indicated that the aflatoxin distribution among laboratory samples taken from a given lot was not symmetrical, but was positively skewed. The experimentally determined mycotoxin distribution among the replicated sample test results (called the observed distribution) had a long tail to the right of the mean, the mean was greater than the median, and the variance was greater than the mean. One theoretical distribution, the negative binomial (NB), stood out among skewed distributions as a good candidate to simulate the characteristics mentioned above in the observed sample to sample distribution (**Ref 18, Bibliography**). The NB is a skewed distribution that allows for a high probability of low counts (particles with little to no mycotoxin) and a low probability of high counts (particles with high levels of a mycotoxin), and the variance has to be greater than the mean. The NB is also used to describe the distribution among particles where contagion is an issue.

A very useful characteristic of the NB distribution is that if the particle to particle distribution is NB with parameters mean C, variance s^2, and shape parameter k, then the sample to sample distribution is also NB with parameters ns*C, ns*s^2, and ns*k where ns is sample size or number of particles in each sample. This characteristic of the NB distribution allows one to measure the variance and mean among

replicate samples of size ns and then compute the particle to particle distribution using the sample size ns as a scale transformation. This is like having a statistical microscope to characterize the particle to particle distribution after measuring the sample to sample distribution (**Ref 17, Bibliography**).

Other skewed type distributions that have provided a good fit to the observed sample to sample distribution are the compound gamma and the log normal distributions (**Ref 19, Bibliography**). The compound gamma is similar to the NB in its ability to simulate extremely skewed sample to sample distributions while the log normal works well for less skewed distributions. For example, the log normal was found to be a suitable distribution to simulate the OTA distribution among samples taken from contaminated lots of green coffee beans (**Ref 20, 21, and 22, Bibliography**).

In most cases, the Mycotoxin Sampling Tool uses the negative binomial distribution to simulate the sample to sample distribution for the granular products listed in **Table A, Annex II**. However, the Lognormal and Normal distribution were used to simulate the sample to sample distribution for aflatoxin and OTA in several products such as green coffee beans and powdered ginger.

7.4 Procedure to calculate accept probabilities (OC curve)

Define a specific mycotoxin sampling plan - As mentioned earlier, a mycotoxin sampling plan is defined by a mycotoxin test procedure and the accept/reject limit, C_a. The mycotoxin test procedure is described by specifying laboratory sample size, ns, test portion size, nss, taken from a comminuted laboratory sample, and number of aliquots, na, quantified by an approved analytical method. The aflatoxin test procedure discussed for shelled corn (maize) in **Annex I and II** will be used as the example to describe the process. In **Annex I** above, an aflatoxin sampling plan is described that uses a

single 1.0 kg laboratory sample of 3000 kernels (ns=3000), a Romer RAS type mill to grind the 1.0 kg laboratory samples of shelled corn (maize), a 50 g test portion (nss=50) taken from the comminuted sample, HPLC to quantify the aflatoxin concentration in a single aliquot (na=1), and an accept/reject limit of 20 ng/g (C_a=20 ng/g). The accept/reject limit is not used in the variance calculation, but is used later to calculate accept probabilities. Once the sampling plan design is specified, the variances are calculated.

Calculate the variances associated with the mycotoxin sampling plan - Using the appropriate sample, sample preparation, and analytical variance equations in **Table A, Annex II**, the total variance, $S^2_{(t)}$, associated with the specific mycotoxin test procedure (given values of ns, nss, and na) can be calculated at a given mycotoxin concentration C by summing the three equations (**Equation I.2, Annex I**) for a range of aflatoxin lot concentrations, C=0, 1, 2, …C_{max}. As an example, the total variance associated with sampling shelled corn (maize) for aflatoxin with the above sampling plan (ns=3000, nss=50, and na=1) are calculated using **Equation I.2** and are shown in **Table I.1** below for selected lot concentration values of C.

Calculation of acceptance probability - Using the appropriate distribution listed in **Table A, Annex II**, the mycotoxin distribution among sample test results, c, taken from a lot with concentration C using a specific sampling plan design (given values of ns, nss, and na) can be calculated knowing the total variance and lot concentration C. Using the method of moments, the parameters of the appropriate distribution are calculated from the total variance, $S^2_{(t)}$, and lot concentration, C. From the distribution of sample test results, the probability P(A) of obtaining a sample concentration, c, less than or equal to the accept/reject limit, C_a , from a lot with concentration C can be calculated. For the aflatoxin-sampling plan for shelled corn (maize) where ns=3000 (1 kg), nss=50, and na=1, the probability of

obtaining a sample concentration, c, less than or equal to the accept/reject limit 20 ng/g (C_a=20 ng/g) is

shown in **Table I.1** for selected lot concentrations ranging from 0 to 70 ng/g. The probability of

rejecting a lot (1-P(A)) with concentration C is also shown in **Table I.1**. The accept and reject

probabilities in **Table I.1** are usually calculated for C values up to C_{max} which is the lot mycotoxin

concentration at which P(A) approached zero. The accept probabilities in **Table I.1** (OC curve) are

plotted in **Figure I.2**.

Table I.1. Accept and reject probabilities (expressed as a %) for an aflatoxin sampling plan for shelled corn using variance equation 2 and the negative binomial distribution. Variance calculated for a 1 kg laboratory sample (ns=3000), Romer mill, 50 g test portion (nss=50), aflatoxin is quantified in one aliquot (na=1) by HPLC methods. Accept/reject limit = 20 ng/g.

Lot Concentration (ng/g)	Total Variance	Probability (%)	
		Accept	Reject
0	0.00	100.00	0.00
1	14.23	99.12	0.88
2	28.66	98.08	1.92
3	43.25	96.88	3.12
4	57.95	95.55	4.45
5	72.76	94.07	5.93
6	87.66	92.46	7.54
7	102.64	90.73	9.27
8	117.69	88.89	11.11
9	132.82	86.94	13.06
10	148.01	84.90	15.10
12	178.56	80.58	19.42
14	209.33	76.00	24.00
16	240.29	71.25	28.75
18	271.43	66.40	33.60
20	302.74	61.53	38.47
22	334.20	56.71	43.29
24	365.80	51.99	48.01
26	397.54	47.42	52.58
28	429.42	43.03	56.97
30	461.41	38.87	61.13
32	493.53	34.94	65.06
34	525.76	31.28	68.72
36	558.09	27.87	72.13
38	590.54	24.74	75.26
40	623.08	21.87	78.13
42	655.72	19.26	80.74
44	688.46	16.89	83.11
46	721.28	14.76	85.24
48	754.20	12.86	87.14
50	787.20	11.16	88.84
55	870.08	7.72	92.28
60	953.44	5.24	94.76
65	1037.28	3.50	96.50
70	1121.55	2.29	97.71

Probability of Accepting Lot of Corn, Shelled (%) for various lot concentrations using sampling plans described in the Edit Plans Tab. For a complete description about operating characteristic curves see Sections 1.2 and 2.3 in the User Guide.
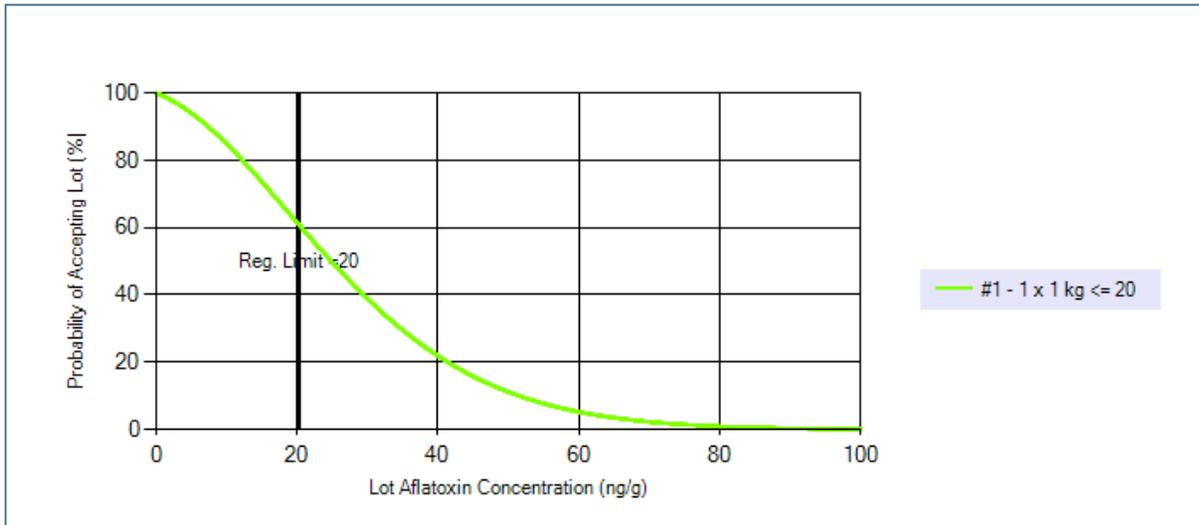

Figure I.2. Operating characteristic curve describing the performance of detecting aflatoxin in shelled corn with a sampling plan that uses 1.0 kg laboratory sample, Romer mill, 50 g test portion, and quantifies aflatoxin in 1 aliquot using HPLC. The accept/reject limit and the regulatory limit are the same at 20 parts per billion (ng/g).

8.0 Annex II Variance Tables

Table A. Summary of sampling studies for various mycotoxins/commodity combinations. References describing each study is shown.

Study #	Mycotoxin	Commodity	References	Variance Sampling (S^2_s)	Variance Sample Preparation (S^2_{sp})	Variance Analytical (Within Lab) (S^2_a)	Mycotoxin Test Procedure Laboratory Sample Size (ns)	Comminuted Test Portion Size (nss)	Number of Aliquots (na)	Concentration (C)	Distribution Among Sample Test Results
1	Aflatoxin	Shelled Peanuts	1, 2, 3, 34	$(10{,}644/ns)9.19C^{1.336}$	$(275/nss)0.294C^{1.729}$	$(1/na)0.083C^{1.654}$	Number of shelled kernels (1,952ker/kg)	Mass (g) Dry Comminution USDA mill powder	Number of aliquots quantified by HPLC from Ref 34	ng/g (ppb) aflatoxin total	Negative Binomial
2	Aflatoxin	Cottonseed	4, 5, 6, 34	$(43{,}200/ns)6.776C^{1.344}$	$(200/nss)0.180C^{1.3508}$	$(1/na)0.086C^{1.567}$	Number of seed (Hull removed) (19,031ker/kg)	Mass (g) Dry Comminution USDA mill powder	Number of aliquots quantified by HPLC from Ref 34	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
3	Aflatoxin	Harvested Inshell Peanuts (Farmer's Stock)	7, 8, 9	$(3713/ns)37.607C^{1.161}$	$(100/nss)2.887C^{1.401}$	$(1/na)0.083C^{1.654}$	Number of inshell pods (882pods/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC from Ref 34	ng/g (ppb) aflatoxin total	Negative Binomial
4	Aflatoxin	Shelled Corn	10, 11, 12	$(3{,}390/ns)11.36C^{0.98}$	$(50/nss)1.254C^{1.27}$	$(1/na)0.143C^{1.16}$	Number of shelled kernels (3,000ker/kg)	Mass (g) Dry Comminution Romer Powder	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin total	Compound Gamma Used Negative Binomial
5	Aflatoxin	Shelled Almonds	13, 14, 15	$(7{,}730/ns)5.759C^{1.561}$	$(100/nss)0.170C^{1.646}$	$(1/na)0.0041C^{1.966}$	Number of shelled kernels (773ker/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
6	Aflatoxin	Inshell Almonds	13, 14, 15	$(7{,}730/ns)5.759C^{1.561}$	$(100/nss)0.170C^{1.646}$	$(1/na)0.0041C^{1.966}$	Number of Inshell Nuts (309nuts/kg) Shell/ker Ratio = 60/40	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
7	Aflatoxin	Shelled Hazelnuts	15, 16, 17	$(10{,}000/ns)4.291C^{1.609}$	$(50/nss)0.021C^{1.545}$	$(1/na)0.0028C^{1.990}$	Number of shelled kernels (1,000ker/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
8	Aflatoxin	Inshell Hazelnuts	15, 16, 17	$(10{,}000/ns)4.291C^{1.609}$	$(50/nss)0.021C^{1.545}$	$(1/na)0.0028C^{1.990}$	Number of Inshell nuts (500Nuts/kg) Shell/Ker Ratio = 50/50	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
9	Aflatoxin	Shelled Pistachios	15	$(8{,}000/ns)7.913C^{1.475}$	$(25/nss)2.334C^{1.522}$	$(1/na)0.0368C^{1.598}$	Number of Shelled Kernels (1,600ker/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
10	Aflatoxin	Inshell Pistachios	15	$(8{,}000/ns)7.913C^{1.475}$	$(25/nss)2.334C^{1.522}$	$(1/na)0.0368C^{1.598}$	Number of Inshell Nuts (800nuts/kg) Shell/Ker Ratio = 50/50	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial

Table A continued.

Study #	Mycotoxin	Commodity	References	Variance			Mycotoxin Test Procedure				Distribution Among Sample Test Results
				Sampling (S^2_s)	Sample Preparation (S^2_{sp})	Analytical (Within Lab) (S^2_a)	Laboratory Sample Size (ns)	Comminuted Test Portion Size (nss)	Number of Aliquots (na)	Concentration (C)	
11	Aflatoxin	Shelled Brazil Nuts	15	$(1{,}850/ns)4.862C^{1.889}$	$(50/nss)0.0306C^{0.632}$	$(1/na)0.0164C^{1.117}$	Number of Shelled Kernels (185ker/kg)	Mass (g) Slurry (Water/Ker 1/1) Comminution	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
12	Aflatoxin	Inshelled Brazil Nuts	15	$(1{,}850/ns)4.862C^{1.889}$	$(50/nss)0.0306C^{0.632}$	$(1/na)0.0164C^{1.117}$	Number of Inshelled Nuts (93Nuts/kg) Shell/Ker Ratio=50/50	Mass (g) Slurry (Water/Ker 1/1) Comminution	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin either total or B1	Negative Binomial
13	Aflatoxin	In Field Ear Corn	18	$(600/ns)8.919C^{2.230}$	$(50/nss)1.254C^{1.27}$	$(1/na)0.143C^{1.16}$	Number of shelled kernels per ear 200 g ker/ear (3,000ker/kg)	Mass (g) Dry Comminution Romer Powder	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin B1	Negative Binomial
14	Aflatoxin	In Field Farmer's Stock Peanuts	19	$(116/ns)17.056C^{1.6686}$	$(100/nss)2.887C^{1.401}$	$(1/na)0.083C^{1.654}$	Number of inshell pods (882pods/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC from Ref 34	ng/g (ppb) aflatoxin total	Negative Binomial
15	Aflatoxin	Powdered Ginger in Capsules	20	$(5/ns)0.138C^{1.0}$	No Test Portion, Entire Sample Extracted	$(1/na)0.0178C^{1.70}$	5 g Laboratory Sample is also the 5 g Test Portion	No Test Portion, Entire Sample Extracted	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin total	Normal
16	Aflatoxin	Powdered Ginger in 1-Lb Bags	21	$(5/ns)4.218C^{1.0}$	No Test Portion, Entire Sample Extracted	$(1/na)0.00349C^{1.70}$	5 g Laboratory Sample is also the 5 g Test Portion	No Test Portion, Entire Sample Extracted	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin total	Normal
17	Aflatoxin	Dried Figs	Not Published	$(590/ns)2.219C^{1.433}$	$(55/nss)0.012C^{1.465}$	$(1/na)0.006C^{1.368}$	Number of dried Figs (59 Figs/kg)	Mass (g) Slurry (Water/Ker 1/1) Comminution	Number of aliquots quantified by HPLC	ng/g (ppb) aflatoxin total	Negative Binomial
18	Fumonisin	Shelled Corn	22, 23, 24	$(3{,}390/ns)0.033C^{1.75}$	$(25/nss)0.011C^{1.59}$	$(1/na)0.014C^{1.44}$	Number of shelled kernels (3,000ker/kg)	Mass (g) Dry Comminution Romer Powder	Number of aliquots quantified by HPLC	ug/g (ppm) Fumonisin either B1, B2, B3 or total	Compound Gamma Used Lognormal
19	Deoxynivalenol (DON)	Shelled Corn	25	$(3{,}000/ns)0.202C^{1.923}$	$(50/nss)0.0193C^{1.140}$	$(1/na)0.0036C^{1.507}$	Number of shelled corn kernels (3,000ker/kg)	Mass (g) Dry Comminution Romer - 25 g	Number of aliquots quantified by Romer - Malone HPLC	ug/g (ppm) DON	Lognormal (not published)
20	Deoxynivalenol (DON)	Wheat	26	$(13{,}620/ns)0.026C^{0.833}$	$(25/nss)0.066C^{0.833}$	$(1/na)0.026C^{0.833}$	Number of raw wheat kernels (30,000ker/kg)	Mass (g) Dry Comminution Romer 25 g	Number of aliquots quantified by Romer FluoroQuant	ug/g (ppm) DON	Lognormal (not published)

Table A Continued.

Study #	Mycotoxin	Commodity	References	Variance			Mycotoxin Test Procedure				Distribution Among Sample Test Results
				Sampling (S^2_s)	Sample Preparation (S^2_{sp})	Analytical (Within Lab) (S^2_a)	Laboratory Sample Size (ns)	Comminuted Test Portion Size (nss)	Number of Aliquots (na)	Concentration (C)	
21	Deoxynivalenol (DON)	Barley	27	$(77,000/ns)0.0122C^{0.947}$	$(50/nss)0.003C^{1.956}$	$(1/na)0.0108C^{1.055}$	Number of raw barley kernels (30,800ker/kg)	Mass (g) Dry Comminution Romer 50 g	Number of aliquots quantified by Romer FluoroQuant	ug/g (ppm) DON	Lognormal (not published)
22	Ochratoxin A (OTA)	Green Coffee Beans	28, 29, 30	$(1,500/ns)1.350C^{1.090}$	$(25/nss)0.272C^{1.646}$	$(1/na)0.008C^{1.605}$	Number of beans (1,500ker/kg)	Mass (g) Dry Comminution VCM Paste	Number of aliquots quantified by HPLC	ng/g (ppb) OTA total	Lognormal
23	Ochratoxin A (OTA)	Powdered Ginger in Capsules	20	$(5/ns)0.108C^{1.0}$	No Test Portion, Entire Sample Extracted	$(1/na)0.00654C^{1.70}$	5 g Laboratory Sample is also the 5 g Test Portion	No Test Portion, Entire Sample Extracted	Number of aliquots quantified by HPLC	ng/g (ppb) OTA total	Normal
24	Ochratoxin A (OTA)	Powdered Ginger in 1-Lb Bags	21	$(5/ns)1.336C^{1.0}$	No Test Portion, Entire Sample Extracted	$(1/na)0.00146C^{1.70}$	5 g Laboratory Sample is also the 5 g Test Portion	No Test Portion, Entire Sample Extracted	Number of aliquots quantified by HPLC	ng/g (ppb) OTA total	Normal
25	Ochratoxin A (OTA)	Oats	Not Published	$(55,796/ns)1.440C^{1.278}$	$(100/nss)0.0074C^{1.638}$	$(1/na)0.0103C^{1.58}$	Number of raw oat kernels (27,898ker/kg)	Mass (g) Dry Comminution Retsch SR300 #20 Screen	Number of aliquots quantified by HPLC	ng/g (ppb) OTA total	Negative Binomial
26	Ochratoxin A (OTA)	Wheat	Not Published	$(60,180/ns)1.557C^{1.132}$	$(5/nss)0.207C^{1.152}$	$(1/na)0.0204C^{1.866}$	Number of raw wheat kernels (30,090ker/kg)	Mass (g) Dry Comminution Retsch SR300 #20 Screen	Number of aliquots quantified by HPLC	ng/g (ppb) OTA total	Negative Binomial
27	FAPAS among lab variability		31			$(1/na)0.0484C^{2.000}$					
28	Horwitz among lab variability (ppb)		32,33			$(1/na)0.2048C^{1.70}$					
29	Whitaker, Horwitz, Analytical Variances - TLC, Immuno, HPLC		34			Among Lab = 2*Within Lab					

Study 3 the sampling variance was calculated by subtracting analytical and sample prep variances from total variances for each of the three (2.26, 4.21, and 6.91 kg) sample sizes.

Studies 13 and 14 measured only total variance. Used sample prep and analytical variances from studies 4 and 3, respectively.

Study 28 analytical variance was determined for various methods, mycotoxins, and commodities using data base from Horwitz Ref 32

References Cited in Table A, Annex II

1. Whitaker, T. B. and Wiser, E. H. 1969. Theoretical investigations into the accuracy of sampling shelled peanuts for aflatoxin. J. Am. Oil Chem. Soc. 46:377-379.

2. Whitaker, T. B. and Dickens, J. W., Monroe, R. J., and Wiser, E. H. 1972. Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J. Am. Oil Chem. Soc. 49:590-593.

3. Whitaker, T. B., Dickens, J. W., and Monroe, R. J. 1974. Variability of aflatoxin test results. J. Am. Oil Chem. Soc. 51:214-218.

4. Velasco, James, Whitaker, T. B., and Whitten, M. E. 1975. Sampling cottonseed lots for aflatoxin contamination. J. Am. Oil Chem. Soc. 52:191-195.

5. Whitaker, T. B., Whitten, M. E., and Monroe, R. J. 1976. Variability associated with testing cottonseed for aflatoxin. J. Am. Oil Chem. Soc. 53:502-505.

6. Whitaker, T. B. and Whitten, M. E. 1977. Evaluation of cottonseed aflatoxin testing programs. J. Am. Oil Chem. Soc. 54:436-441.

7. Whitaker, T. B., Dowell, F. E., Hagler, W. M., Jr., Giesbrecht, F. G., and Wu, J. 1994. Variability Associated with sampling, sample preparation, and chemical testing of farmers' stock peanuts. J. Assoc. Off. Anal. Chem., Int., 77:107-116.

8. Whitaker, T. B., Giesbrecht, F. G., Wu, J., Hagler, W. M., Jr., and Dowell, F. E. 1994. Predicting the distribution of aflatoxin test results from farmers' stock peanuts. J. Assoc. Off. Anal. Chem., Int., 77:659-666.

9. Whitaker, T. B., Wu, J., Dowell, F. E., Hagler, W. M., Jr., and Giesbrecht, F. G. 1994. Effects of sample size and sample acceptance level on the number of aflatoxin-contaminated farmers' stock lots accepted and rejected at the buying point. J. Assoc. Off. Anal. Chem., Int., 77:1672-1680.

10. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., Young, J. H., and Bowman, D. T. 2000. Testing shelled corn for aflatoxin, Part I: Estimation of variance components. J. Assoc. Off. Anal. Chem., Int., 83:1264-1269.

11. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., and Young, J. H.. 2000. Testing shelled corn for aflatoxin, Part II: Modeling the distribution of aflatoxin test results. J. Assoc. Off. Anal. Chem., Int., 83:1270-1278.

12. Johansson, A. S., Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., Young, J. H. and Bowman, D. T. 2000. Testing shelled corn for aflatoxin, Part III: Evaluating the performance of aflatoxin sampling plans. J. Assoc. Off. Anal. Chem., Int., 83:1279-1284.

13. Whitaker, T.B., Slate, A.B., Jacobs, M., Hurley, J.M., Adams, J.G., and Giesbrecht, F.G. 2006. Sampling almonds for aflatoxin, Part I: Estimation of uncertainty associated with sampling, sample preparation, and analysis. J. Assoc. Off. Anal. Chem., Int., 89:1027-1034.

14. Whitaker, T.B., Slate, A.B., Hurley, J.M., and Giesbrecht, F.G. 2006. Sampling almonds for aflatoxin, Part II: Estimating risk associated with various sampling plan designs. J. Assoc. Off. Anal. Chem., Int., 90:778-785.

15. Food and Agriculture Organization, Codex Alimentarius Commission, 2010. Report of the 4th Session of the Codex Committee on Contaminants in Foods, Izmir, Turkey, April 26-30 April 2010, Alinorm 10/33/41.

16. Ozay, G., Seyhan, F., Yilmaz, A. Whitaker, T.B., and Slate, A.B. 2006. Sampling hazelnuts for aflatoxin, Part I: Estimation of uncertainty associated with sampling, sample preparation, and analysis, J. Assoc. Off. Anal. Chem., Int., 89:1004-1011.

17. Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T.B., Slate, A.B., and Giesbrecht, F.G. 2007. Sampling hazelnuts for aflatoxin: Effect of sample size and accept/reject limits on risk of misclassifying lots. J. Assoc. Off. Anal. Chem., Int., 90:1028-1035.

18. Brera, C., De Santis, B., Prantera, E., Debegnach, F., Pannunzi, E., Fasano, F., Berdini, C., Slate, A.B., Miraglia, M., and. Whitaker, T.B. 2010. The effect of sample size when evaluating "*In-Field*" sampling plans for aflatoxin AFB_1 determination in corn. J. Ag. and Food Chem., 58:8481-8489.

19. Whitaker, T. B., J. W. Dorner, F. G. Giesbrecht, A. B. Slate. 2004. Variability Among Aflatoxin Test Results on Runner Peanuts Harvested from Small Field Plots. Peanut Science, 31:59-63.

20. Trucksess, M.W., Whitaker, T.B., Weaver, C.M., Slate, A., Giesbrecht, F.G., Rader, J.I., and Betz, J.M. 2009. Sampling and analytical variability associated with the determination of total aflatoxins and ochratoxin A in powdered ginger sold as a dietary supplement in capsules. J Agric. Food Chem., 57:321-325.

21. Whitaker, T.B., Trucksess, M.W., Weaver, C.M., and Slate, A.B. 2009. Sampling and analytical variability associated with the determination of aflatoxin and ochratoxin A in bulk lots of powdered ginger marketed in 1-lb bags. J. Anal. Bioanal. Chem., 395:1291-1299.

22. Whitaker, T. B., Trucksess, M., Johansson, A. S., Giesbrecht, F. G., Hagler, W. M., Jr., Bowman, D. T. 1998. Variability associated with testing corn for fumonisin. J. Assoc. Off. Anal. Chem., Int., 81:1162-1168.

23. Whitaker, T. B., Hagler, Jr., W. M., Johansson, A. S., Giesbrecht, F. G., and Trucksess, M. W. 2001. Sampling shelled corn for fumonisin. IN: *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium*, Eds: W. J. deKoe, R. A. Samson, H. P. van Egmond, J. Gilbert, and M. Sabino. IUPAC, Hazekamp 2, 6707 HG Wageningen, The Netherlands. pp 97-107.

24. Whitaker, T.B., Doko, M. B., Maestroni, B. M., Slate, A. B., and Ogunbanwo, B. F. 2007. Evaluating the performance of sampling plans to detect fumonisin B_1 in maize lots marketed in Nigeria. J. Assoc. Off. Anal. Chem., Int., 90:1050-1059.

25. Whitaker, T.B., Richard, J.R., Giesbrecht, F.G., Slate, A.B., and Ruiz, N. 2003. Estimating deoxynivalenol in shelled corn barge lots by measuring deoxynivalenol in corn screenings. J. Assoc. Off. Anal. Chem., Int., 86:1187-1192.

26. Whitaker, T. B., Hagler, Jr., W. M., Giesbrecht, F. G., and Johansson, A. S. 2000. Sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. J. Assoc. Off. Anal. Chem., Int. 83:1285-1292.

27. Freese, L., Friedrich, R., Kendall, D., and Tanner, S. 2000. Variability of Deoxynivalenol measurements in Barley. J. Assoc. Off. Anal. Chem., Int., 83:1259-1263.

28. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., Franca, R.C.A. 2004. Testing green coffee for ochratoxin A, Part I: estimation of variance components, J. Assoc. Off. Anal. Chem., Int., 87:884-891.

29. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., and Franca, R.C.A. 2005. Testing green coffee for ochratoxin A, Part II: observed distribution of ochratoxin A test results, J. Assoc. Off. Anal. Chem., Int., 88:780-787.

30. Vargas, E.A., Whitaker, T.B., Santos, E.A., Slate, A.B., Lima, F.B., and Franca, R.C.A. 2006. Testing green coffee for ochratoxin A, Part III: Performance of ochratoxin A sampling plan, J. Assoc. Off. Anal. Chem., Int., 89:1021-1026.

31. Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. J. Royal Society of Chemistry, 125:385-386.

32. Horwitz, W., Albert, R., and Nesheim. 1993. Reliability of Mycotoxin Assays-An update. J. Assoc. Off. Anal. Chem., Int., 76:461-491.

33. Horwitz, W. and Albert, R. 2006. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J. Assoc. Off. Anal. Chem., Int., 89:1095-1109.

34. Whitaker, T. B., Horwitz, W., Albert, R., and Nesheim, S. 1996. Variability associated with methods used to measure aflatoxin in agricultural commodities. J. Assoc. Off. Anal Chem., Int., 79:476-485.