Introduction

Mycotoxins are toxic and/or carcinogenic compounds produced by various fungal species that grow on various agricultural commodities (Cullen and Newberne, 1994). There are many different mycotoxins (CAST, 2003) and a partial list is shown in Table 1.

Table 1. Partial listing of several mycotoxins produced by various fungi.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Fumonisins</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Deoxynivalenol</td>
</tr>
<tr>
<td>Patulin</td>
<td>Zearalenone</td>
</tr>
</tbody>
</table>

Commodities can be contaminated either in the field or in storage. Pre- and post-harvest strategies to prevent crop contamination include yearly crop rotations, irrigation in hot and dry weather, use of pesticides to reduce the insect population, drying crops to a safe moisture level, and providing protective storage (Phillips et al., 1994). Because mycotoxins are toxic and carcinogenic in animals, many countries regulate the maximum level that can occur in foods and feeds. Most regulations are concerned with controlling aflatoxin because it is considered the most toxic and carcinogenic of the naturally occurring mycotoxins. A recent FAO/WHO survey indicated that almost 100 countries regulate aflatoxin in foods and feeds (FAO, 1995). However, maximum levels differ widely from country to country because of a lack of agreement on what constitutes a safe maximum level for humans. Some of the maximum levels found in the FAO/WHO survey for aflatoxin are shown below in Table 2 (FAO, 1995).

Table 2. Examples of aflatoxin legal limits found in various countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Aflatoxin B$_1$ (ppb)</th>
<th>Total aflatoxin (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>EU</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Egypt</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Maize</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Peanuts</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Nigeria</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

It is important to be able to detect and quantify the mycotoxin concentration in foods and feeds destined for human and animal consumption. In research, quality assurance, and regulatory activities, correct decisions concerning the fate of commercial lots can only be made if the mycotoxin concentration in the lot can be determined with a high degree of accuracy and precision. The mycotoxin concentration of a bulk lot is usually estimated by measuring the mycotoxin concentration in a small portion of
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the lot or a sample taken from the lot (Figure 1).

Is the lot/batch concentration the same as the sample?
Is the sample concentration equal to or below the stated limit?

Lot/batch (50,000 kg)
Sample (2 kg)

Figure 1. Lot mycotoxin concentration is assumed to equal the mycotoxin concentration measured in a small sample.

The mycotoxin concentration in the bulk lot is assumed to be the same as the measured mycotoxin concentration in the sample. Based on the measured sample concentration, some decision is made about the edible quality of the bulk lot or the effect of a treatment or a process on reducing aflatoxin in the lot. For example, in a regulatory environment, decisions will be made to classify the lot as acceptable or unacceptable based upon a comparison of the measured sample concentration to a legal limit. If the sample concentration does not accurately reflect the lot concentration, then the lot may be misclassified and there may be undesirable economic and/or health consequences. Fortunately, sampling plans can be designed to minimise the misclassification of lots and reduce the undesirable consequences associated with regulatory decisions about the fate of bulk lots. In this chapter, sampling plans will be defined, sources of uncertainty associated with a mycotoxin sampling plan will be identified, risks associated with misclassifying lots will be discussed, and methods that reduce misclassification of lots will be described.

Definition of sampling plan

A mycotoxin sampling plan is defined by a mycotoxin test procedure and a defined accept/reject limit. A mycotoxin test procedure is a multi-stage process (Figure 2) and generally consists of three steps: sampling, sample preparation, and analysis (quantification).

Lot/batch

Sample (2 kg)

Sample preparation

Analysis

Test result

Test procedure

Figure 2. A mycotoxin test procedure usually consists of a sampling, sample preparation and analytical step.

The sampling step specifies how the sample will be selected or taken from the bulk lot and the size of the sample. For granular products, the sample preparation step is also a two-part process where the sample is ground in a mill to reduce particle size and a subsample is removed from the comminuted sample. Finally in the analytical step, the mycotoxin is solvent-extracted from the comminuted subsample and quantified using approved procedures.

The measured mycotoxin concentration in the sample is used to estimate the true mycotoxin concentration in the bulk lot or compared to a defined accept/reject limit that is usually equal to a maximum level or regulatory legal limit. Comparing the measured concentration to an accept/reject limit is often called acceptance sampling because the measured concentration value is not as important as whether the measured concentration (and thus the lot concentration) is above or below a legal limit. In quality assurance and research activities, a precise and accurate estimate of the true lot mycotoxin concentration becomes important.
UNCERTAINTY

There is always some level of uncertainty associated with a sampling plan. Because of the uncertainty associated with a mycotoxin sampling plan, the true mycotoxin concentration of a bulk lot cannot be determined with 100% certainty; nor can all lots be correctly classified into good and bad categories (based upon some legal limit) with 100% accuracy. Accuracy and precision are two types of uncertainties associated with a sampling plan (Cochran and Cox, 1957).

Accuracy

Accuracy is defined as the closeness of measured values to the true value. Another term associated with accuracy is bias. A bias is some force or influence that makes the measured values deviate from the true value in a consistent manner on the average. Using target practice as an example, the center of the target is analogous to the true value and holes in the target represent the measured values (Figure 3). Figure 3 shows that the rifle used on the left is not as accurate as the rifle used on the right where the average of the cluster of shots is around the center of the target.

Mathematically, accuracy ($A$) is the difference between the true value ($U$) and the average of the $n$ measured values ($X$) (Equation 1).

$$A = U - \frac{\text{SUM}(X)}{n} \quad (1)$$

Precision

Precision is defined as the closeness of measured values to each other. Another term for precision is variability. The definition of precision makes no mention about how close the measured values are to the true value. Using target practice to illustrate precision, the closeness of the holes to each other is a measure of precision (Figure 4).

Three statistical measures of variability, variance ($V$), standard deviation ($S$), or coefficient of variation (CV) can be used as a measure of precision ($P$) (Equations 2-4).

$$V = \frac{\sum (x_i-m)^2}{n-1} \quad \text{for } i = 1,2,\ldots,n $$ \quad (2)

$$S = \sqrt{V} \quad (3)$$

The CV, expressed as a percent:

$$CV = 100 \times \left(\frac{S}{m}\right) \quad (4)$$
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Where \( x_i \) is the measured value and \( m \) is the mean of the \( n \) \( x_i \) values. Precision is associated with variability, which can occur with each step of the mycotoxin test procedure.

When describing the uncertainty of a process, one must consider the various combinations of accuracy and precision that may occur. As shown in Figure 5, there are four extreme combinations of accuracy and precision: low precision and low accuracy, low precision and high accuracy, high precision and low accuracy, and high precision and high accuracy.

![Figure 5. The four extreme combinations of uncertainty that can occur with a sampling plan.](image)

The worst possible situation is to have a process with low precision and low accuracy. The best possible situation is to have a process that has both high precision and high accuracy. The goal associated with detecting a mycotoxin in a bulk shipment is to design a mycotoxin test procedure or sampling plan that has both high precision and high accuracy.

**Sample selection**

Procedures used to take a sample from a bulk lot are extremely important. Every individual item in the lot should have an equal chance of being chosen (called random sampling). Biases are introduced by sample selection methods if equipment and procedures used to select the sample prohibit or reduce the chances of any item in the lot from being chosen. Examples of bias in the sample selection process, shown in Figure 6, are the use of a sampling probe that does not allow larger particles into the probe, a probe that does not reach every location in the shipment, and use of a single probing point in a poorly mixed lot.

![Figure 6. Different types of biases associated with selecting samples from bulk lots. (1) Particles larger than probe opening; (2) Some particles cannot be reached; (3) Using a single probing point with an unmixed lot.](image)

If the lot has been blended thoroughly from the various material handling operations, then the contaminated particles are assumed to be distributed uniformly throughout the lot (Williams, 1991). In this situation, it is probably not too important from what location in the lot the sample is drawn. However, if the lot is contaminated because of moisture leaks that cause high moisture clumps or for other localized reasons, then the mycotoxin-contaminated particles may be located in isolated pockets in the lot (Shotwell et al., 1975). If the sample is drawn from a single location, the contaminated particles may be missed or too many contaminated particles may be collected (Figure 7).
Non-homogeneous and homogeneous distribution

Because contaminated particles may not be distributed uniformly throughout the lot, the sample should be an accumulation of many small portions taken from many different locations throughout the lot (Bauwin and Ryan, 1982; Hurburgh and Bern, 1983). FAO/WHO recommends that each incremental portion be about 200 g and one incremental portion be taken for every 200 kg of product (FAO, 2001). The accumulation of many small incremental portions is called a bulk sample. If the bulk sample is larger than desired, the bulk sample should be blended and subdivided until the desired sample size is achieved (Figure 8).

The smallest sample size that is subdivided from the bulk sample and comminuted in a grinder in the sample preparation step is called the test sample. It is generally more difficult to obtain a representative (lack of bias) test sample from a lot at rest (static lot) than from a moving stream of the product (dynamic lot) as the lot is moved from one location to another. Sample selection methods differ depending on whether the lot is static or dynamic.

STATIC LOTS

Examples of static lots are commodities contained in storage bins, rail cars, or many small containers such as sacks. When drawing a sample from a bulk container, a probing pattern should be developed so that product can be collected from different locations in the lot. An example of several probing patterns used by the USDA to collect samples from peanut lots is shown in Figure 9 (USDA, 1975; Parker et al., 1982; Whitaker and Dowell, 1995).

The sampling probe should be long enough to reach the bottom of the container when possible. Attempts should be made to use a sampling rate similar to the 200 g per 200 kg mentioned above. However, it may not be possible to achieve the suggested sampling rate because of the design of the sampling equipment, size of the individual containers, and the size of the lot. As an example, a test sample (TSS) of 5,000 g is to be taken from a lot (LS) of 25,000 kg. The preferred increment size (ISS) is 200 g. The minimum number of increments needed to provide a test sample of 5,000 g is TSS/ISS or 25 incremental portions of 200 g each. If a total of 25 incremental portions are to be taken from the lot of 25,000 kg, then an increment is taken for every 1,000 kg (25,000/25) of lot (taking an increment every 1,000 kg of lot is larger than the recommended 200 kg of lot). If a 200 g incremental portion is taken every 200 kg of lot, then a total of 125 (25,000/200) increments of 200 g each will be taken and the bulk sample size is 25,000 g or five times bigger than the needed test sample size of 5,000 g. The 25,000 g
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Figure 9. Example of several 5- and 8-probe patterns used by the US Department of Agriculture to sample large peanut containers (trucks and wagons) for grade.

x = 5 Probe Patterns
x + 0 = 8 Probe Patterns

g bulk sample must be subdivided to obtain the 5,000 g test sample. A flow diagram showing the interactions among all the variables is shown in Figure 10.

When sampling a static lot in separate containers such as sacks or retail containers, the sample should be taken from many containers dispersed throughout the lot. When storing sacks in a storage facility, access lanes should be constructed in order to allow access to sacks at interior locations. The recommended number of containers sampled can vary from one in four in small lots (<20 metric tonnes) to the square root of the total number of containers for large (>20 metric tonnes) lots (FAO, 2001).

If the lot is in a container where access is limited, the sample should be drawn when the product is either being removed from or being placed into the container. If the accumulated bulk sample is larger than required, the bulk sample should be thoroughly blended and reduced to the required test sample size using a suitable divider that randomly removes a test sample from the bulk sample.

DYNAMIC LOTS

True random sampling can be more nearly achieved when selecting a bulk sample from a moving stream as the product is transferred (i.e. conveyor belt) from one location to another. When sampling from a moving stream, small increments of product should be taken along the entire length of the moving stream (Figure 11). Small increments of product should be taken across the entire cross section of the moving stream; composite all the increments of product to obtain a bulk sample. If the bulk sample is larger than required, then blend and subdivide the bulk sample to obtain the desired size test sample.
Is the number of increments (Nl) greater than the preferred number of increments (PNI)?

**YES**

Test sample size = Nl x ISS  
(Nl = TSS/ISS)

**No**

Use Preferred Number of Increments (PNI)  
(PNI = Lot size/Incremental lot size)

Bulk sample size (BSS) = PNI x Increment size (ISS)

Divide

Test sample size (TSS)

Figure 10. Interaction between lot size, increment size, and test sample size.

Automatic sampling equipment such as cross-cut samplers (Figure 12) are commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals.

When automatic equipment is not available, a person can be assigned to manually pass a cup though the stream at periodic intervals to collect the bulk sample. Whether using automatic or manual methods, small increments of product should be collected and composited at frequent and uniform intervals throughout the entire time product flows past the sampling point.

Figure 11. Sample selection from a moving stream of product should be the accumulation of many small incremental portions taken from the beginning to the end of the product stream.

Figure 12. The automatic sampler cup should move at a constant velocity and cut through the entire stream of product.
Cross-cut samplers should be installed in the following manner: (a) the plane of the opening of the sampling cup should be perpendicular to the direction of flow; (b) the sampling cup should pass through the entire cross sectional area of the stream; and (c) the opening of the sampling cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the sampling cup opening should be two to three times the largest dimensions of the items in the lot.

The size of the bulk sample, $S$ in kg, taken from a lot by a cross-cut sampler is

$$S = \frac{(D)(L)}{(T)(V)},$$

where $D$ is the width of the sampling cup opening in cm, $L$ is the lot size in kg, $T$ is interval or time between cup movement through the stream in seconds, and $V$ is cup velocity in cm/sec.

Equation 5 can also be used to compute other terms of interest such as the time between cuts, $T$. For example, the required time, $T$, between cuts of the sampling cup to obtain a 10 kg sample from a 30,000 kg lot where the sampling cup width is 5.08 cm (2 inches), and the cup velocity through the stream 30 cm/sec. Solving for $T$ in Equation 5,

$$T = \frac{(5.08 \text{ cm} \times 30,000 \text{ kg})}{(10 \text{ kg} \times 30 \text{ cm/sec})} = 508 \text{ sec}$$

If the lot is moving at 1000 kg per minute, the entire lot will pass through the sampler in 30 minutes and only three or four cuts will be made by the cup through the lot. This may be considered too infrequent, because too much product passes the sampling point between the times the cup cuts through the stream. The interaction among the variables in Equation 5 needs to be fully understood in terms of the amount of sample accumulated and the frequency of cuts through the product.

**BULK VERSUS TEST SAMPLE**

Because contaminated particles may not be uniformly dispersed, many incremental portions are taken from many different locations throughout the lot and accumulated to form a bulk sample. As a result, the bulk sample is usually larger than the desired test sample size used to estimate the lot mycotoxin concentration. For granular material, the test sample is the smallest sample of granular product ground in a mill in the sample preparation step. For finely ground materials (corn flour) or liquids (milk), the test sample is the smallest sample used in the analytical step to quantify the mycotoxin. When the bulk sample is larger than the test sample, mechanical dividers such as a Boerner or riffle divider should be used to remove the desired test sample from the bulk sample. Mechanical dividers are considered to produce random divisions (Parker et al., 1982); therefore, the bulk sample does not have to be blended before the test sample is removed. However, if the test sample is to be removed from the bulk sample using quartering or a manual device such as a cup or scoop, then the bulk sample should be blended before the test sample is removed.

If the test sample is a granular product such as shelled corn or nuts, then the test sample should not be further reduced in size before grinding the sample in the sample preparation step. As the test sample becomes smaller, the uncertainty associated with estimating the true lot mycotoxin concentration becomes greater. As will be shown later, the size of the test sample put through the grinder should be as large as possible. Recommended sample sizes for various commodities are shown in Table 3.

**Sample preparation**

Once a sample has been taken from the lot, the test sample must be prepared for mycotoxin quantification. Since it is not practical to extract the mycotoxin from a large test sample, the mycotoxin is usually extracted from a much smaller portion of product (subsample) taken from the test sample. If the commodity is a granular product such as shelled corn, it is
Table 3. Product sample sizes used by the United States Food and Drug Administration.

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Package type</th>
<th>Lot size</th>
<th>Number of sample units</th>
<th>Unit size</th>
<th>Sample (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut butter</td>
<td>Smooth</td>
<td>Consumer &amp; bulk</td>
<td>24</td>
<td>0.5</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Crunchy, butter, raw roasted,</td>
<td>Consumer &amp; bulk</td>
<td>12</td>
<td>1</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>ground topping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree nuts</td>
<td>In-shell, shelled</td>
<td>Consumer &amp; bulk</td>
<td>48</td>
<td>1</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>slices or flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>paste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil nuts</td>
<td>In-shell in import status</td>
<td>Bulk</td>
<td>&lt;200 bags</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Pistachio nuts</td>
<td>In-shell in import status</td>
<td>Bulk</td>
<td>201-800 bags</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>801-2000 bags</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Corn</td>
<td>Shelled, meal, flour dirt</td>
<td>Consumer &amp; bulk</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>Peanut, cottonseed</td>
<td>Bulk</td>
<td>15</td>
<td>4</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Oilseed meals</td>
<td>Pumpkin, melon, sesame etc</td>
<td>Bulk</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Edible seeds</td>
<td></td>
<td>Consumer &amp; bulk</td>
<td>50</td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Ginger root</td>
<td>Dried, whole ground</td>
<td>Bulk &amp; 'n' units</td>
<td>( \sqrt{n} )</td>
<td>10</td>
<td>10 × 0.06</td>
<td>15</td>
</tr>
<tr>
<td>Milk</td>
<td>Whole, low fat, skim</td>
<td>Consumer &amp; bulk</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bulk &amp; consumer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small grains</td>
<td>Sorghum, wheat, barley etc</td>
<td>Bulk</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Dried fruit</td>
<td>i.e. figs</td>
<td>Consumer &amp; bulk</td>
<td>50</td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mixtures</td>
<td>Commodity particles</td>
<td>Consumer &amp; bulk</td>
<td>50</td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>large</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commodity particles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>finely ground</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Essential that the entire test sample be comminuted in a suitable mill before a subsample is removed from the test sample (Dickens and Whitaker, 1982; Campbell et al., 1986). Removing a subsample of whole seed from the test sample before the comminuting process would eliminate the benefits associated with the larger size test sample of granular product. After the test sample has been comminuted, a subsample is removed from the comminuted test sample for mycotoxin extraction (Figure 13).

Grinders should be used that reduce the particle size of the seed in the test sample to the smallest size possible. Grinders that produce small particles provide a more homogeneous test sample (Figure 13). As a result the mycotoxin concentration of the subsample will more closely reflect the true mycotoxin concentration of the test sample. Some grinders such as the Romer mill (Malone, 2000) and the USDA peanut mill (Dickens and Satterwhite, 1969) are designed to automatically provide a subsample during the grinding process. If the mill does not provide a subsample, the subsample can be obtained using a riffle divider. If the subsample is obtained using a manual device such as a scoop, blend the comminuted test sample before scooping out a subsample.

Normally, there will be no sample preparation step associated with samples of non-granular products such as liquids (milk) or paste...
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Figure 13. A test sample of granular product should be ground in a mill to reduce particle size.

(peanut butter). A small portion of the sample may have to be removed for mycotoxin analysis because the entire sample cannot be analysed. However, it is important to blend or mix liquid samples and paste samples before removing a small portion for mycotoxin analysis.

Subsample sizes vary, but usually are on the order of 25 to 1000 g depending on particle size. The smaller the particle size, the smaller the subsample size can be without increasing error or uncertainty.

Analytical quantification

Once the subsample is removed from the ground test sample, the mycotoxin is extracted by blending a solvent with the comminuted subsample. Before the mycotoxin can be quantified in the solvent extract, analytical methods usually consist of several steps related to removing interfering compounds (i.e. oils) and concentrating the mycotoxins for quantification. These steps include centrifugation, filtration, drying, and dilution (Steyn et al., 1991). Three types of methods can be used to quantify the mycotoxin extracted from the subsample: thin layer chromatography (TLC), ELISA methods that use antibody technology, and high performance liquid chromatography (HPLC). Organizations such as the Association of Official Analytical Chemists (AOAC) evaluate the performance of analytical methods using collaborative studies.

There are several sources of bias associated with analytical methods. Less than 100% of the mycotoxin may be extracted from the subsample by the solvents; compounds other than mycotoxins may be extracted into the solvent and mistakenly quantified as a mycotoxin; mycotoxin standards used in quantification may not be exact; and instruments to measure the mycotoxin may not be correctly calibrated.

Accept/reject limit

Once the mycotoxin concentration is quantified, the sample value is used to estimate the true shipment concentration or is compared to an accept/reject limit (ARL). The ARL is a predefined threshold value, usually equal to a legal limit used in regulatory applications. If the sample mycotoxin value is less than or equal to the ARL, the lot is accepted. If otherwise, the lot is rejected. When lots are inspected by regulatory agencies, the ARL is usually set equal to the legal limit. However, manufacturers of consumer-ready products will often use an ARL less than the legal limit to reduce the chances that consumer-ready products will be found by regulatory agencies with mycotoxin concentrations above the legal limit. Often private industry will use an ARL that is about half the legal limit.

Many countries agree on the need to establish legal limits, but often disagree on the value of the limit. A survey by FAO in 1995 (FAO, 1995) showed that some countries have aflatoxin legal limits based upon $B_1$ only and some countries use total ($B_1 + B_2 + G_1 + G_2$) aflatoxin and these legal limits vary widely. The CODEX Committee on Food Additives and Contaminates has established a standard
aflatoxin limit for peanuts at 15 ppb total aflatoxin for peanuts traded on the international market (FAO, 2001). This limit does not infringe on any nation’s internal limits.

**Random variation**

Even when using accepted sampling, sample preparation, and analytical procedures (Campbell et al., 1986; AOAC, 1990; Nesheim, 1979), there are errors (the term error will be used to denote variability) associated with each of the above steps of the mycotoxin test procedure (Whitaker et al., 1974). Because of these errors, the true mycotoxin concentration in the lot cannot be determined with 100% certainty by measuring the mycotoxin concentration in a test sample taken from the lot. For example, 10 replicated aflatoxin test results from each of six contaminated shelled peanut lots are shown in Table 4 (Whitaker et al., 1972). For each test result in the table, the mycotoxin test procedure consisted of (a) comminuting a 5.45 kg test sample of peanut kernels in a USDA subsampling mill developed by the US Department of Agriculture (Dickens and Satterwhite, 1969), (b) removing a 280 g subsample from the comminuted test sample, (c) solvent extracting aflatoxins from a 280 g subsample as described by AOAC Method II (AOAC, 1990), and (d) quantifying the aflatoxins densitometrically using thin layer chromatography (TLC). The 10 aflatoxin test results from each lot are ranked from low to high to demonstrate several important characteristics about replicated aflatoxin test results taken from the same contaminated lot.

First, the wide range among replicated sample results from the same lot reflects the large variability associated with estimating the true mycotoxin content of a bulk lot. In Table 4, the variability is described by the standard deviation (SD) and the coefficient of variation (CV). The maximum sample result can be four to five times the lot concentration (the average of the 10 sample results is the best estimate of the lot concentration). Secondly, the amount of variation among the 10 sample results appears to be a function of the lot concentration. As the lot concentration increases, the standard deviation among sample results increases, but the standard deviation relative to the lot mean, as measured by the CV, decreases. Thirdly, the distribution of the 10 sample results for each lot in Table 4 is not always symmetrical about the lot concentration. The distributions are positively skewed, meaning that more than half of the sample results are below the true lot concentration. However, the distribution of sample test results becomes more symmetrical as the lot concentration increases. This skewness can be observed by counting the number of aflatoxin test results above and below the lot concentration in Table 4 (average of the 10 sample test results). If a single sample is tested

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**Table 4. Distribution of aflatoxin test results for ten 5.4 kg samples from each of six lots of shelled peanuts**

<table>
<thead>
<tr>
<th>Lot number</th>
<th>Sample test result (ng/g)</th>
<th>Mean (ppb)</th>
<th>SD (ppb)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 0 0 0 2 4 8 14 28 43</td>
<td>10</td>
<td>15</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>0 0 0 3 13 19 41 43 69</td>
<td>19</td>
<td>24</td>
<td>126</td>
</tr>
<tr>
<td>3</td>
<td>0 6 6 8 10 50 60 62 66 130</td>
<td>40</td>
<td>42</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>5 12 56 66 70 92 98 132 141 164</td>
<td>84</td>
<td>53</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>18 50 53 72 82 108 112 127 182 191</td>
<td>100</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>29 37 41 71 95 117 168 174 183 197</td>
<td>111</td>
<td>66</td>
<td>59</td>
</tr>
</tbody>
</table>

*a Whitaker et al., 1972.
b Aflatoxin test results are ordered by aflatoxin concentration, (ng/g).
c SD = Standard Deviation
d CV = Coefficient of Variation = SD\(\times\)100/mean
Sampling feeds for mycotoxin analysis

from a contaminated lot, there is more than a 50% chance that the sample test result will be lower than the true lot concentration. While it cannot be shown in Table 4, the skewness is greater for small sample sizes and the distribution becomes more symmetrical as sample size increases (Whitaker et al., 1972). The above characteristics described by Table 4 for aflatoxin in peanuts are also generally found for other mycotoxins and other commodities (Dickens et al., 1979; Whitaker et al., 1993; Whitaker et al., 1998).

The sources of the variability among mycotoxin test results in Table 4 are associated with each step of the mycotoxin test procedure. The sampling, sample preparation, and analytical steps of the mycotoxin test procedure each contribute to the total variability observed among mycotoxin test results. As shown in Figure 14, the total error or variability is the sum of the sampling, sample preparation, and analytical variability.

![Figure 14](image)

**Figure 14.** Total error of the mycotoxin test procedure is the sum of sampling, sample preparation, and analytical errors.

Among the statistical measures of variability shown in Equations 2 to 4, only the variance is additive. Therefore, it is assumed that the total variance associated with a mycotoxin test procedure is the sum of the sampling, sample preparation, and analytical variances (Equation 6).

\[
VT = VS + VSS + VA
\]  

(6)

Reasons why each step of the mycotoxin test procedure contributes to the overall variability are discussed below. An example of the magnitude of the contribution each step contributes to the total variability is also shown when testing shelled corn (maize) for aflatoxin.

**SAMPLING VARIABILITY**

Studies by researchers on a wide variety of agricultural products (peanuts, cottonseed, shelled corn and pistachio nuts) indicate that, especially for small sample sizes, the sampling step is usually the largest source of variability associated with the mycotoxin test procedure (Dickens et al., 1979). Even when using accepted sample selection equipment and random sample selection procedures, sampling error is large because of the extreme distribution among contaminated particles within a lot. Studies by researchers on a wide variety of agricultural products such as peanuts and shelled corn (Johansson et al., 2000a; Cucullu et al., 1986; Cucullu et al., 1977) indicate that a very small percentage (0.1%) of the kernels in the lot are contaminated and the concentration on a single kernel may be extremely high. Cucullu et al., (1986) reported aflatoxin concentrations in excess of 1,000,000 ng/g (ppb) for individual peanut kernels and 5,000,000 ng/g for cottonseed. Shotwell et al. (1974) reported finding over 400,000 ng/g aflatoxin in a corn kernel.

Because of this extreme range in aflatoxin concentrations among a few contaminated kernels in a lot, variation among replicated sample test results tends to be large. As an example, the sampling variance, VS, associated with testing shelled corn was estimated empirically (Johansson et al., 2000a) and is shown in Equation 7 for any sample size, ns.

\[
VS = (12.95/ns) M^{0.98}
\]  

(7)

where \( M \) is the aflatoxin concentration in the lot in nanograms of total aflatoxin per g of corn, ns is the mass of shelled corn in the sample in
kg (kernel count per gram was 3.0). From Equation 7 one can see that the sampling variance is a function of the lot aflatoxin concentration M and sample size ns. The sampling variance among replicated 0.91 kg (2 lb) samples taken from a lot of shelled corn at 20 ppb is 268.1. The coefficient of variation is 81.8%.

Researchers have developed equations to describe the sampling variance for several commodities and mycotoxins (Whitaker et al., 1974; 1993; 1998; Johansson et al., 2000a). The equations are specific for the type of mycotoxin and the type of product studied, but generally show that sampling variance increases with an increase in concentration, and decreases with an increase in sample size.

SAMPLE PREPARATION VARIABILITY

Once the test sample has been taken from the lot, the sample must be prepared for mycotoxin quantification. Since it is not practical to extract the mycotoxin from a large test sample, the test sample is comminuted in a mill and the mycotoxin is extracted from a small subsample taken from the comminuted test sample. If the commodity is a granular product such as shelled corn, it is essential that the entire test sample be comminuted in a suitable mill before a subsample is removed from the test sample (Campbell et al., 1986). Removing a subsample of whole seed from the test sample before the comminuting process is simply a sample size reduction process and eliminates the benefits associated with the larger size sample of granular product. After the sample has been comminuted in a mill to reduce particle size, a subsample is removed for mycotoxin extraction. It is assumed that the mycotoxin distribution of contaminated particles in the comminuted sample is similar to the distribution among contaminated kernels found in the lot. As a result, there is also variability among replicated subsamples taken from the same test sample. However, the sample preparation variance is not as large as the sampling variance due to the large number of comminuted particles in the subsample. An example of sample preparation variance for aflatoxin and shelled corn, VSS, is shown below in Equation 8 for any subsample size ns (Johansson et al., 2000a).

\[ VSS = \frac{62.70}{n_{ss}} M^{1.27} \]  

where M is the aflatoxin concentration in the test sample in ppb, ns is the mass of shelled corn in the subsample in grams. The variance in Equation 8 also reflects the use of a Romer mill that produces a particle size where most of the particles will pass through a No. 20 screen. From Equation 8, it can be seen that the sample preparation variance is also a function of the aflatoxin concentration in the sample and the subsample size. The sample preparation variance associated with a 50 g subsample taken from a sample at 20 ppb is 56.3 and the CV is 37.5%.

Researchers have developed equations to describe the sample preparation variance for several commodities, mills, and mycotoxins (Whitaker et al., 1993; 1998; Johansson et al., 2000a). The equations are specific for the type of mycotoxin, type of mill (particle size), and the type of product used in the study. The type of mill affects the particle size distribution. If the average particle size decreases (number of particles per unit mass increases), then the subsampling variances for a given size subsample decreases.

ANALYTICAL VARIABILITY

Once the subsample is removed from the comminuted test sample, the mycotoxin is solvent-extracted. Analytical methods usually involve several steps such as solvent extraction, centrifugation, drying, dilution, and quantification (Steyn et al., 1991). As a result, there can be considerable variation among replicated analyses on the same subsample extract. The analytical variance, VAh, associated with HPLC techniques used to measure aflatoxin in shelled corn is given by Equation 9 (Whitaker et al., 1996) for any number of aliquots, na.
Sampling feeds for mycotoxin analysis

\[ VA_h = \frac{0.143}{na} M^{1.16} \]  

(9)

where \( M \) is the aflatoxin concentration in the subsample in ppb, \( na \) is the number of aliquots quantified by HPLC methods. The analytical variance and CV associated with using HPLC to measure aflatoxin in a comminuted subsample of corn at 20 ng/g, is 4.6 and 10.7%, respectively.

High performance liquid chromatography tends to have less variability than other analytical technologies such as TLC and immunoassay (ELISA) methods (Whitaker et al., 1996). Using precision estimates from collaborative studies, the analytical variances associated with TLC (\( V_A_t \)) and ELISA (\( V_A_e \)) methods to measure aflatoxin in corn are shown in Equations 10 and 11, respectively.

\[ V_A_t = \frac{0.316}{na} M^{1.744} \]  

(10)

\[ V_A_e = \frac{0.631}{na} M^{1.293} \]  

(11)

The coefficients of variation associated with measuring aflatoxin in a corn subsample at 20 ppb with the TLC and ELISA methods are 38.3 and 27.5%, respectively. The variability associated with HPLC, 10.7%, (Equation 9) is lower than either TLC or ELISA.

All of the analytical variance information described above reflects results from single laboratories and does not reflect among-laboratory variances. As a result, some laboratories may have higher or lower variances than those reported in Equations 9, 10, and 11. Among-laboratory variance is about double the within-laboratory variance (Whitaker et al., 1996).

**TOTAL VARIABILITY**

As shown in Table 5 and Equation 6, the total variability, \( VT \), (using variance as the statistical measure of variability) associated with a mycotoxin test procedure is equal to the sum of the sampling (\( VS \)), sample preparation (\( V_S S \)), and analytical (\( VA \)) variances associated with each step of the mycotoxin test procedure. The total variability associated with testing shelled corn for aflatoxin, grinding the test sample in a Romer mill, and quantifying aflatoxin by immunoassay is the sum of Equations 7, 8, and 11 (Equation 12).

\[ VT = \left( \frac{12.95}{ns} \right) M^{0.98} + \left( \frac{62.70}{nss} \right) M^{1.27} + \left( \frac{0.631}{na} \right) M^{1.293} \]  

(12)

**Table 5. The variability measured by the variance associated with a 0.91 kg sample, 50 g subsample, measuring aflatoxin in 1 aliquot by immunoassay in a lot of shelled corn at 20 ppb aflatoxin.**

<table>
<thead>
<tr>
<th>Variance</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample = 0.91 kg</td>
<td>268.1</td>
</tr>
<tr>
<td>Subsample, 50 g</td>
<td>56.3</td>
</tr>
<tr>
<td>Immunoassay, 1 aliquot</td>
<td>30.4</td>
</tr>
<tr>
<td>Total</td>
<td>354.8</td>
</tr>
</tbody>
</table>

1 Sampling, sample preparation, and analysis errors account for about 75.5, 15.9, and 8.6% of the total error, respectively.

2 Romer mill used to grind

Using Equation 12, the total, sampling, sample preparation, and analytical variances associated with testing shelled corn over a range of lot concentrations (\( M \)) when using a 0.91 kg sample (\( ns \)), grinding the test sample in a Romer mill, taking a 50 g subsample (\( nss \)) from a comminuted sample, and quantifying aflatoxin in one aliquot (\( na \)) by immunoassay methods are shown in Figure 15.

When sampling a shipment of shelled corn at 20 ppb, the magnitude of the variance associated with each step of the above aflatoxin test procedure (Equation 12) is shown below in Equation 13.

\[ VT = 268.1 + 56.3 + 30.4 = 354.8 \]  

(13)

As shown in Table 5, the sampling, subsampling, and analytical variances account for 75.5, 15.9, and 8.6% of the total mycotoxin testing variance, respectively.

As the above example demonstrates, the sampling step accounts for most of the variability (uncertainty) associated with the total variability of a mycotoxin test procedure because of the
Figure 15. Variability of each step of the aflatoxin test procedure, as measured by the variance (V), increases with aflatoxin concentration. The total variance, VT, is the sum of sampling variance, VS, sample preparation variance, VSS, and analytical variance, VA.

Reducing variability of a mycotoxin test procedure

The only way to achieve a more precise estimate of the true lot concentration is to reduce the total variability of the test procedure. The total variability of the test procedure can be reduced by reducing the variability associated with each step of the mycotoxin test procedure. Increasing the size of the sample can reduce the sampling variability. The sample preparation variability can be reduced either by increasing the size of the subsample and/or by increasing the degree of comminuting (increasing the number of particles per unit mass in the subsample). The analytical variance can be reduced by either increasing the number of aliquots quantified by the analytical method and/or using a more precise quantification method (i.e. using HPLC instead of TLC). If the variability associated with one or more of these steps can be reduced, then the total variability associated with a mycotoxin test result can be reduced (Equation 6).

Decreasing the total variability (improving precision) associated with a mycotoxin test procedure will decrease the range of possible aflatoxin test results when replicated tests are made on the same lot. The range of mycotoxin test results associated with any size sample and subsample, and number of analyses about the lot concentration M can be estimated from the total variance, VT, or standard deviation, S, (square root of the total variance) associated with the mycotoxin test procedure. Approximately 95% of all test results will fall between a low of \((M - 1.96 \times S)\) and a high of \((M + 1.96 \times S)\).
As an example, when sampling a lot of shelled corn at 20 ppb using a 0.91 kg sample (ns), grinding the test sample in a Romer mill, taking a 50 g subsample (nss) from a comminuted sample, and quantifying aflatoxin in one aliquot (na) using an immunoassay method, Equation 13 shows that the total variance and standard deviation are 354.8 and 18.8, respectively. The range of aflatoxin test results should fall between 20 ± (1.96 x 18.8) or 20 ± 37 or 0 and 57 ppb (Table 5).

The calculated range of aflatoxin test results is only valid for a normal distribution where test results are symmetrical about the mean. The distribution among aflatoxin test results is usually skewed, but will approach a symmetrical distribution as sample size becomes large.

SAMPLE SIZE

The effect of increasing sample size on reducing the total variability and the range of mycotoxin test results when testing a contaminated lot of shelled corn at 20 ppb aflatoxin is shown in Table 6 when increasing sample size from 0.91 to 4.54 kg.

Table 6. Effect of increasing sample size on reducing the sampling variability.

<table>
<thead>
<tr>
<th>Sample size (kg)</th>
<th>0.91 kg</th>
<th>4.54 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance</td>
<td>266.5</td>
<td>53.3</td>
</tr>
<tr>
<td>Subsample², 50 g</td>
<td>56.3</td>
<td>56.3</td>
</tr>
<tr>
<td>TLC, 1 aliquot</td>
<td>27.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Total</td>
<td>350.7</td>
<td>137.5</td>
</tr>
<tr>
<td>Range</td>
<td>20 ± 37</td>
<td>20 ± 23</td>
</tr>
</tbody>
</table>

¹Lot shelled corn at 20 ppb
²Sample size = 0.91 kg
³Romer mill used to grind

The range of aflatoxin test results is reduced from 20 ± 37 to 20 ± 23 ppb as sample size is increased from 0.91 to 4.54 kg.

SUBSAMPLE SIZE

The effect of increasing subsample size from 50 to 100 g on reducing the sample preparation variance is shown in Table 7.

Table 7. Effect of increasing subsample size on reducing sample preparation variability.

<table>
<thead>
<tr>
<th>Subsample size (g)</th>
<th>50 g</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance</td>
<td>266.5</td>
<td>266.5</td>
</tr>
<tr>
<td>Subsample³</td>
<td>56.3</td>
<td>28.2</td>
</tr>
<tr>
<td>TLC, 1 aliquot</td>
<td>27.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Total</td>
<td>350.7</td>
<td>322.6</td>
</tr>
<tr>
<td>Range</td>
<td>20 ± 37</td>
<td>20 ± 36</td>
</tr>
</tbody>
</table>

¹Lot shelled corn at 20 ppb
²Sample size = 0.91 kg
³Romer mill used to grind

The sample preparation variance is cut in half and is reduced from 56.3 to 28.2. The total variance is reduced from 350.7 to 322.6. The range of aflatoxin test results is reduced from 20 ± 37 to 20 ± 36.

NUMBER OF ALIQUOTS QUANTIFIED

The effect of increasing number of aliquots quantified in the analytical step from 1 to 2 on reducing the analytical variance for immunoassay type method is shown in Table 8.

The analytical variance is cut in half and is reduced from 27.9 to 14.0. The total variance is reduced from 350.7 to 336.8. The reduction is so small that the range of aflatoxin test results is not significantly affected.

There are different costs associated with reducing the variability of each step of a
mycotoxin test procedure. One needs to try and maximize the variance reduction for a given cost. Increasing sample size is usually the best use of resources when reducing the total variability of mycotoxin test results.

Table 8. Effect of increasing number of aliquots quantified for aflatoxin on reducing analytical variability\(^1\).

<table>
<thead>
<tr>
<th>Number of aliquots</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance(^2)</td>
<td>266.5</td>
<td>266.5</td>
</tr>
<tr>
<td>Subsample = 50 g</td>
<td>56.3</td>
<td>56.3</td>
</tr>
<tr>
<td>TLC</td>
<td>27.9</td>
<td>14.0</td>
</tr>
<tr>
<td>Total</td>
<td>350.7</td>
<td>336.8</td>
</tr>
<tr>
<td>Range</td>
<td>20 ± 37</td>
<td>20 ± 37</td>
</tr>
</tbody>
</table>

\(^1\)Lot shelled corn at 20 ppb
\(^2\)Sample size = 0.91 kg

Designing mycotoxin sampling plans

Because of the variability among mycotoxin test results, two types of mistakes are associated with any mycotoxin sampling plan. First, good lots (lots with a concentration less than or equal to the legal limit) will test bad and be rejected by the sampling plan. This type of mistake is often called the seller's risk (false positives) since these lots will be rejected at an unnecessary cost to the seller of the product. Secondly, bad lots (lots with a concentration greater than the legal limit) will test good and be accepted by the sampling plan. This type of mistake is called the buyer's risk (false negatives) since contaminated lots will be processed into feed or food causing possible health problems and/or economic loss to the buyer of the product. In order to maintain an effective regulatory and/or quality control program, the above two risks associated with a sample design must be evaluated (Figure 16). Based upon these evaluations, the costs and benefits (benefits refers to removal of mycotoxin contaminated lots) associated with a sampling program need to be evaluated.

A lot is termed bad when the sample test result \(X\) is above some predefined ARL \(X_c\) and the lot is termed good when \(X\) is less than or equal to \(X_c\). While \(X_c\) is usually equal to the legal limit \(M_c\), \(X_c\) can be greater than or less than \(M_c\). For a given sample design, lots with a mycotoxin concentration \(M\) will be accepted with a certain probability \(P(M) = \text{prob}(X < X_c | M)\) by the sampling plan. A plot of \(P(M)\) versus the lot concentration \(M\) is called an **operating characteristic (OC)** curve. Figure 17 depicts the general shape of an OC curve.

As \(M\) approaches 0, \(P(M)\) approaches 1 or 100%, and as \(M\) becomes large, \(P(M)\) approaches zero. Lots with little to no contamination (\(M = 0\)) are accepted by the sampling plan 100% of the time; lots with very high levels of contamination (\(M = \text{large}\)) are never accepted (rejected 100% of the time) by the sampling plan; lots with contamination levels near the accept/reject limit are accepted by the sampling plan less than 100% of the time. The shape of the OC curve is uniquely defined for a particular sampling plan design with designated values of sample size, degree of comminution, subsample size, type of analytical method, and number of analyses, and the accept/reject limit \(X_c\).
CALCULATION OF ACCEPTANCE PROBABILITY

The acceptance probability $P(M)$ associated with sampling a commodity for a mycotoxin can be computed if the distribution among replicated sample test results can be described and if the appropriate variance relationships are known for a mycotoxin test procedure. Several skewed distributions such as the negative binomial and compound gamma have been shown to adequately describe the observed mycotoxin distribution of sample test results for several commodities and several mycotoxins. An example of the probability of accepting and rejecting shelled corn lots over a range of lot concentrations for the sampling plan where $ns = 4.54$ kg, Romer mill, $nss = 50$ g, immunoassay analytical method, $na = 1$ aliquot, and accept/reject limit $X_c = 20$ ppb is shown in Table 9.

Table 9 shows that most of the lots below 5 ppb are accepted by the sampling plan and most of the lots above 60 ppb are rejected by the sampling plan. For example, 95% and 2% of the lots at 5 and 60 ppb are accepted by the sampling plan, respectively. As lot concentration $M$ increases, the percentage lots accepted by the sampling plan decreases. The acceptance probabilities in Table 9 are plotted in Figure 18 and a smooth curve forced through the points.

For a given sampling plan, the OC curve indicates the magnitudes of the buyer’s and seller’s risk. When $M_c$ is defined as the legal limit or the maximum lot concentration...
acceptable, lots with $M > M_c$ are bad and lots with $M = M_c$ are good. In Figure 17, the area under the OC curve for $M > M_c$ represents the buyer’s risk (bad lots accepted) while the area above the OC curve for $M < M_c$ represents the seller’s risk (good lots rejected) for a particular sampling plan. Using the example in Table 9, if lots at 20 ppb or less are considered good lots and those greater than 20 ppb are considered bad lots, then lots rejected below 20 ppb are considered a measure of the seller’s risk (good lots rejected) and the lots accepted above 20 ppb are considered the buyer’s risk (bad lots rejected).

Because the shape of the OC curve is uniquely defined by the sample size, degree of comminution, subsample size, the number of analyses and the accept/reject limit, these parameters can be used to reduce the buyer’s and seller’s risks associated with a sampling plan.

SAMPLE SIZE EFFECT ON RISKS

The effect of increasing sample size on the shape of the OC curve when testing shelled corn lots for aflatoxin is shown in Figure 19 where the accept/reject limit is equal to the legal limit of 20 ppb. As sample size increases from 0.91 to 9.07 kg, the slope of the OC curve about the legal limit increases forcing the two areas associated with each risk to decrease. As a result, increasing the sample size decreases both the buyer’s and seller’s risks. The same effect can be obtained by increasing either the degree of sample comminution, subsample size or number of analyses.

ACCEPT/REJECT LIMIT EFFECTS ON RISKS

The effect of changing the ARL, relative to the legal limit, on the two risks when testing shelled corn lots for aflatoxin is shown in Figure 20. If the legal limit is assumed to be 20 ppb, then changing $X_c$ to a value less than 20 ppb shifts the OC curve to the left. Compared to the sampling plan where $X_c = 20$, the buyer’s risk decreases, but the seller’s risk increases. If $X_c$ becomes larger than 20, the OC curve shifts

![Figure 18. Operating characteristic curve for a sampling plan that uses a 4.54 kg sample, Romer mill, 50 g subsample, ELISA method, 1 aliquot, and a 20 ppb (ng/g) accept/reject limit.](image-url)
Sampling feeds for mycotoxin analysis

Figure 19. Three operating characteristic curves showing that increasing sample size reduces both buyer’s and seller’s risks.

Figure 20. Three operating characteristic curves for three accept/reject limits. If the accept/reject limit (10 ng/g) is less than the legal limit (20 ng/g), the seller’s risk increases and the buyer’s risk decreases. If the accept/reject (30 ng/g) is greater than the legal limit (20 ng/g), the seller’s risk decreases and the buyer’s risk increases.

to the right. As a result, the seller’s risk decreases but the buyer’s risk increases. Changing the ARL relative to the legal limit can reduce only one of the two risks, because reducing one risk will automatically increase the other risk.

Methods have been developed to predict the seller’s and buyer’s risks, the total number of lots accepted and rejected, the amount of mycotoxin in the accepted and rejected lots, and the costs associated with mycotoxin inspection programmes for several commodities (FAO, 1993; Whitaker and Dickens, 1979; Johansson et al., 2000c; Whitaker et al., 1995). These methods have been used by the USDA/AMS and the peanut industry to design aflatoxin testing programmes for shelled peanuts.
MULTIPLE SAMPLES

Increasing the number of samples of a given size taken from a contaminated lot can reduce the risks associated with classifying lots. If the mycotoxin among all samples is averaged, the effect is the same as that described in Figure 19 for the effect of increasing sample size. However, if all multiple sample test results are required to test less than some ARL, the effect is the same as shown in Figure 20 for changing the ARL relative to the legal limit. Three sampling plans showing the effect of requiring either one, two, or three 4.54 kg samples to all test less than or equal to 20 ppb is shown in Figure 21.

As the number of samples required to test less than or equal to the ARL increases, the OC curve shifts to the left reducing the buyer’s risk but increasing the seller’s risk. The result is similar to reducing the ARL. This type of sampling plan is often used late in the marketing system on finished product destined for animals or humans that have little chance of product containing mycotoxin above the legal limit. The buyer is placing most of the risk on the seller.

Conclusions

Because of the uncertainties (biases and variability) associated with a mycotoxin test procedure, it is difficult to determine with 100% certainty the true concentration of a bulk lot. Even when the sample is correctly selected (no biases), there will be variability associated with the mycotoxin test procedure. The variance associated with a mycotoxin test procedure is the sum of sampling, sample preparation, and analytical variances. For small sample sizes, sampling is usually the largest source of variability. Increasing sample size, the degree of sample comminution, subsample size, and the number of aliquots quantified can reduce the variability associated with a mycotoxin test procedure. Reducing variability of the mycotoxin test procedure will reduce the number of lots misclassified by the sampling plan.

![Figure 21](image)

**Figure 21.** Three operating characteristic curves showing the effect of requiring either one, two, or three 4.54 kg samples of shelled corn to all test less than or equal to the accept/reject limit of 20 ng/g total aflatoxin to accept the lot.
References


