

Chapter 14

SAMPLING METHODS TO MEASURE AFLATOXIN AND GRADE FACTORS OF PEANUTS

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INTRODUCTION

Various grade factors and aflatoxin concentration of peanut lots marketed in the U.S. are measured several times during the marketing process from the farm to the consumer (Davidson *et al.*, 1982). A simplified flow chart in Fig. 1 gives an indication where in the marketing process peanuts are usually graded and tested for aflatoxin. The flow chart does not indicate where shellers and manufacturers grade and test peanuts as part of their own "in-house" quality assurance program.

Each grade factor and the aflatoxin concentration of a lot are estimated from samples taken from the lot. Because of random variation associated with sampling, sample preparation, and measurement steps, the true value of each grade factor and the true aflatoxin concentration of the lot cannot be determined with 100% certainty. Accurate and precise estimates of the true grade factors and the true aflatoxin concentration of the lot are important since poor estimates can cause large economic losses to farmers, shellers, and manufacturers and possible health risks to the consumer.

In this chapter we will attempt to identify where errors occur in the sampling, sample preparation, and measurement procedures; predict the magnitude of the errors; and suggest methods to reduce the errors associated with estimating grade factors and aflatoxin concentrations of peanuts.

SAMPLE SELECTION

Procedures used to take a sample from a peanut lot are extremely important. Every individual item in the peanut lot should have an equal chance of being chosen (called random sampling). Biases will be introduced by the 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 (Gy, 1982).

If the items of interest in a peanut lot (i.e., foreign material, loose shelled kernels, etc.) are uniformly dispersed throughout the lot, a sample can be taken from any single, randomly chosen location within the lot. However, selecting a sample from a lot where the items of interest are not homogeneously dispersed throughout the lot requires that the sample (sometimes called the

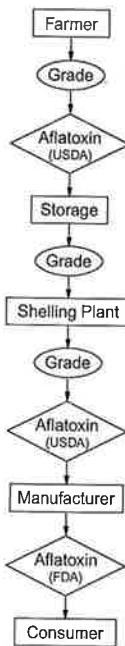


Fig. 1. Schematic diagram showing location in the marketing chain where peanut lots are sampled to estimate grade and aflatoxin concentration.

bulk sample) be the accumulation of many small portions or increments of product selected from different locations throughout the lot. If the bulk sample is larger than desired, the bulk sample should be blended and subdivided until the desired sample size is achieved. The smallest size sampling unit used to estimate a lot characteristic is often called the test sample. If there is a question about the items being uniformly dispersed throughout the lot, one should take the conservative approach and accumulate many small increments taken from different locations throughout the lot.

Static Lots

A static lot can be defined as a large mass of peanuts contained either (a) in a single large container such as a wagon, truck, or railcar or (b) in many small containers such as sacks or boxes and the peanuts are stationary at the time a sample is selected. An example of a static lot is peanuts brought to the buying point by a farmer either in a drying wagon or a truck. Selecting a truly random sample from a static lot can be difficult because the container may not allow access to all items and the items may be physically segregated in the lot due to differences in size and density.

Taking a bulk sample from a static lot usually requires the use of probing devices to select product from the lot. The probing devices are specially designed for the type of container. The probe should (a) be long enough to

reach all product, (b) not restrict any item in the population from being selected, and (c) not alter the items in the lot. As mentioned above, the bulk sample should be composite from many small increments of product taken from many different location throughout the lot. Therefore, the product should be probed in different randomly chosen locations.

The number of incremental portions taken from a lot is usually directly proportional to lot size. As will be shown later, the United States Department of Agriculture (USDA) suggests a using about one probe per metric ton for bulk containers.

Dynamic Lots

True random sampling can be more nearly achieved when selecting a bulk sample from a moving stream of peanuts as the lot is transferred, for example, by a conveyor belt from one location to another. When sampling from a moving stream, take small increments of product from the entire length of the moving stream; composite the product to obtain a bulk sample; if the bulk sample is larger than the required size, then blend and subdivide the bulk sample to obtain the desired size test sample.

Automatic sampling equipment such as cross-cut samplers (Fig. 2) are

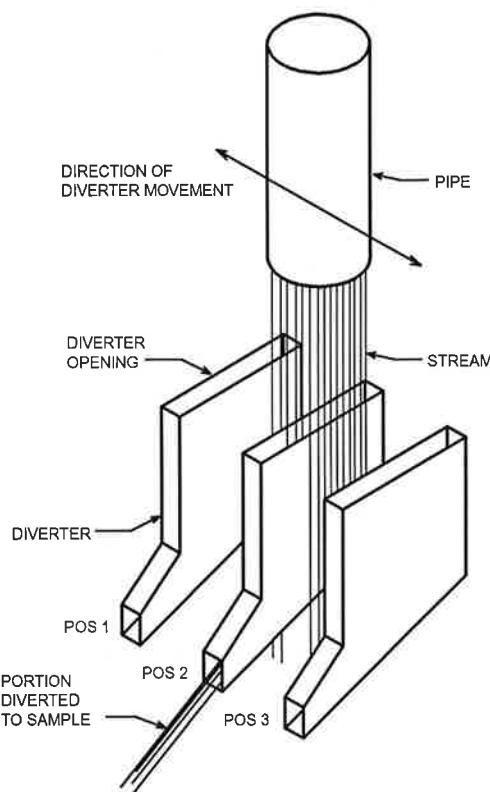


Fig. 2. Schematic diagram showing cross-cut sampler and a moving stream of peanuts.

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 composite at frequent and uniform intervals throughout the entire time peanuts flow past the sampling point.

Cross-cut samplers should be installed in the following manner: (a) the plane of the opening of the diverter cup should be perpendicular to the direction of flow, (b) the diverter cup should pass through the entire cross sectional area of the stream, and (c) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter 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:

$$S = (D)(L) / (T)(V) \quad \text{Eq. (1)}$$

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

Equation 1 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 diverter cup to obtain a 10-kg sample from a 30,000-kg lot where the diverter cup width is 5.08 cm (2 inches), and the cup velocity through the stream 30 cm/sec. Solving for T in Eq. 1:

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

If the lot is moving at 1000 kg/min, 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, in that too much product passes through the sampler between the time the cup cuts through the stream. The interaction among the variables in Eq. 1 needs to be fully understood in terms of the amount of sample accumulated and the frequency of taking product.

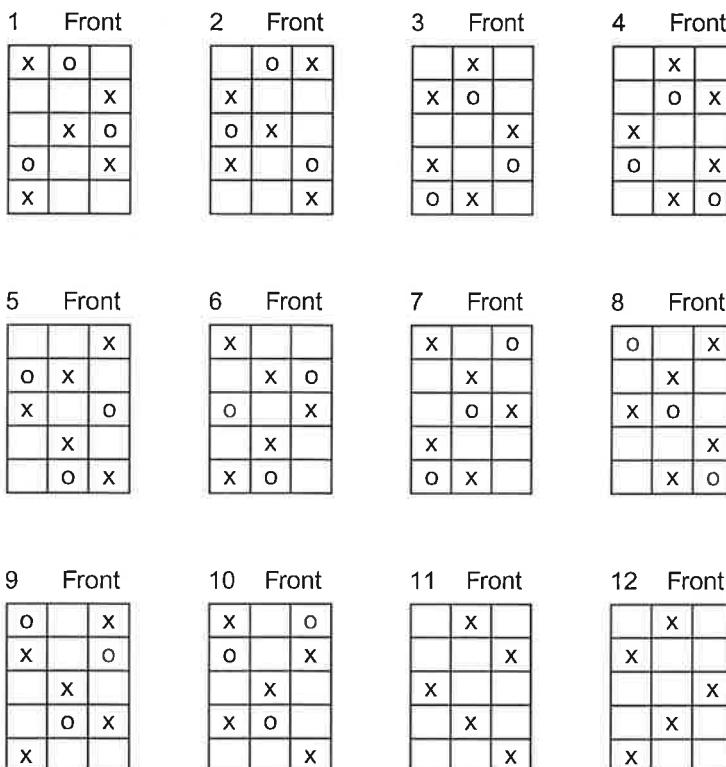
Sampling Farmers Stock Peanuts

When a farmer brings peanuts to a buying point, a sample is removed from the lot and graded by the Federal State Inspection Service (FSIS) of the USDA. The support price a farmer receives for the peanuts is determined from a loan schedule that uses the various grade factors measured from the sample. Farmers usually take peanuts to the buying point in a wagon or truck. The static lot is sampled with a pneumatic sampler which takes cores 10 cm in diameter from top to bottom of the lot. The number of cores taken depends on the lot size. A minimum of five cores are taken from lots below 5443 kg and a maximum of 16 cores are taken from lots over 13,608 kg. The USDA uses a series of different probing patterns to insure that different patterns are

used from lot to lot (USDA, 1991). An example of several five- and eight-probe patterns used by USDA for farmers stock lots is shown in Fig. 3. Depending upon the depth of the peanuts in a wagon or truck, a core contains about 4.5 kg of peanut pods, loose shelled kernels, and foreign material. Regardless of the number of cores taken, the material from each core is composited, the bulk sample is blended and subdivided so that at least a 2-kg test sample can be graded for support price and visually inspected for an aflatoxin-producing fungi.

Sampling Milled Peanuts

About 40,000 lots of milled peanuts are sampled each crop year to measure grade factors and aflatoxin concentration. Unlike farmers stock peanuts, milled peanut lots can be sampled as the lot is conveyed from one location to another inside the shelling plant. Sampling milled peanut lots from a moving stream is accomplished with automatic sampling equipment



x = 5 Probe Patterns

x + o = 8 Probe Patterns

Fig. 3. Example of several five- and eight-probe patterns used by the USDA to sample farmers stock peanuts for grade and support price.

where shelling plant facilities can accommodate installation. Ideally, automatic sampling equipment has a diverter cup that cuts completely through the peanut stream. The USDA specifies that the width of the diverter cup open on automatic sampling equipment has a minimum width of 3.8 cm (1.5 inches). The frequency of cup movement through the peanut stream is set so the cup passes through the peanut stream at least every 225 to 365 kg of peanuts (USDA, 1993). Automatic sampling equipment is placed at the end of the processing line. Care must be exercised in not splitting whole kernels in the sampling process.

If sampling from a moving stream cannot be achieved, milled peanut lots are sampled in the container. Special devices are used by the FSIS to probe the container and remove peanuts. Recommended procedures require that a bulk sample of milled peanuts be accumulated by probing about 1/4 of the container in a lot. About 70 kg are taken from each lot. The bulk sample is subdivided to obtain a 66-kg test sample for aflatoxin analysis and a 4-kg test sample for grade analysis.

Sampling Cleaned In-Shell Peanuts

Most valencia-, 35% of virginia-, and some runner-type peanuts are cleaned and marketed in the shell. U.S. grade standards exist only for virginia-type in-shell peanuts, but these standards are used as guidelines when grading valencia- and runner-type in-shell peanuts. As with milled peanuts, cleaned in-shell peanuts are sampled either from a moving stream or by probing the container. Since the pod is larger than a kernel, the minimum dimension of the diverter cup on automatic sampling equipment should be about 6.75 cm (3 inches) to 7.0 cm (4 inches). The USDA also recommends that in-shell peanuts samples be taken from about one in every 10 containers.

GRADE FACTORS

Lot grade factors are estimated by measuring the grade factor in a sample taken from the lot (Davidson *et al.*, 1982). Even when samples are correctly selected and no biases are introduced by the sample selection procedure, there may still be variation among grade factors measured from replicate samples taken from the same lot of peanuts. Table 1 shows the variability for several grade factors and support price measurements using 16 grade samples taken from the same farmers stock lot of runner-type peanuts. The total variability, as measured by variance, VT, is the sum of sampling variance, VS, and analysis or measurement variance, VA:

$$VT = VS + VA \quad \text{Eq. (2)}$$

The sampling variability is a result of the distribution among the kernel characteristic of interest in the lot. For example, kernel diameter varies from kernel to kernel in the lot. Davidson *et al.* (1978) described the distribution of kernel diameters in a lot by the logistic distribution. If a single sample containing n kernels is taken from a lot with no selection bias, then the

average kernel diameter among n kernels can be measured and used as an estimate of the average kernel diameter in the lot. If N samples of n kernels are taken from the same lot, the grade factor will vary from sample to sample (Table 1). Sampling variability is inversely proportional to sample size, n . The larger the sample size, the smaller the variability.

The measurement error is a function of the equipment and skill of the grader performing the measurement. Using kernel diameter again as an example, if the same peanut kernel sample was measured repeatedly using USDA screens, the measured kernel diameter would vary among the repeated measurements. Measurement variability can be reduced by improving equipment design, properly maintaining equipment, improving the skill of the graders, and not overloading the equipment with too big a sample.

Farmers Stock Peanuts

Davidson *et al.* (1982) and Dickens and Johnson (1987) described the grading process for farmers stock peanuts. In summary, when a farmer brings a peanut lot to the buying point, an 1800-g sample is removed (using procedures described previously) and graded by USDA. The 1800-g grade sample is separated into foreign material, loose shelled kernels, and pods. The percentage by weight of foreign material (%FM) and loose shelled

Table 1. Several grade factors each measured from 16 USDA grade samples taken from the same farmers stock lot of runner peanuts.

Sample	FM ^a	LSK	SMK	SS	OK	DAM	Support price
No.	% -----					\$	
1	5	4	64	4	11	2	587.81
2	7	5	61	3	13	2	558.22
3	6	4	62	3	12	1	566.05
4	6	4	63	4	11	1	581.50
5	5	3	62	4	11	3	572.26
6	6	4	64	4	11	1	589.90
7	7	4	62	4	12	1	573.28
8	5	4	64	3	11	1	582.67
9	5	3	64	4	10	1	594.67
10	5	4	63	4	10	3	574.62
11	7	4	61	6	11	3	572.10
12	6	4	64	3	12	2	579.59
13	7	4	63	4	11	2	577.08
14	7	4	62	4	12	4	562.75
15	7	4	64	6	9	1	601.31
16	10	5	63	3	12	1	564.77
Mean	6.3	4.0	62.9	3.4	11.1	1.8	577.41

^aThe foreign material and loose shelled kernels were measured using 1800-g samples. The remaining grade factors were measured using a 500-g pod sample.

kernels (%LSK) in the 1800-g sample are determined. A 500 g-test sample of pods is removed from the in-shell portion of the 1800-g sample. The 500-g test sample of pods is shelled and the percentage by weight of sound mature kernels (%SMK), sound splits (%SS), other kernels (%OK), damaged kernels (%DAM), extra large kernels (%ELK), and moisture content on a % wet basis, (%M) are determined. The grade factor %ELK only applies to virginia-type peanuts. The support price or quota loan value (QLV) the farmer receives for his peanut lot is determined from a USDA loan schedule that uses the above grade factors to calculate the QLV. An example of a loan schedule and how the QLV is computed from the grade factors is described by Davidson *et al.* (1982).

Three independent studies by Whitaker *et al.* (1991), Davidson *et al.* (1990), and Dowell (1992) measured the total variability (VT) associated with measuring each grade factor and QLV using the specified USDA grade sample size of farmers stock peanuts. Dowell (1992) also measured the variability with a second sample size that was double the official USDA grade sample size (3600 g for LSK and FM and 1000 g for the remaining grade factors). Using two sample sizes allowed the total variance to be partitioned into sampling variance (VS) and measurement variance (VA) components.

The VA values for each of the above grade factors were about the same for all three studies. The studies indicated the variance was a function of the magnitude of the grade factor. Whitaker *et al.* (1991) described the relationship between the variance and the mean grade factor using binomial relationships. If p is the fraction of defective items in the lot, then the mean number of defective items in a sample of n items is np and the variance among replicated samples of n items is $np(1-p)$. Actual variance measurements for several grade factors support the binomial assumption because the variance increases as the mean values increase from zero to 50% and the variance decreases as the mean continues to increase from 50 to 100%.

Because grade factors are reported in percent (by weight), it is helpful to express the binomial relationships for variance as follows:

$$VT = (a)(m) - [(a/100)(m^2)] \quad \text{Eq. (3)}$$

where the coefficient 'a' is equal to $100/n$ and m is the mean grade factor in percent. The coefficient a was determined from regression techniques and is shown in Table 2 for each grade factor.

Equation 3 and coefficients 'a' in Table 2 can be used to estimate the variance associated with several grade factors when using the official farmers stock grade sample. If a lot contains 5% FM, 5% LSK, 65% SMK, 5% SS, 7% OK, and 1% DAM, the variance for each grade factor is 0.79, 0.47, 2.62, 1.0, 1.1, and 0.36, respectively. The above variance values can be used to estimate the range of grade factors expected when using a 1800-g sample to measure FM and LSK and the 500-g sample to measure the remaining grade factors. Approximately 95% of all test results will fall between a low of $(m - 1.96 \cdot SD)$ and a high of $(m + 1.96 \cdot SD)$, where SD is the standard deviation or the square root of the variance, VT , given in Eq. 3. The two expressions are only valid for a normal distribution where test results are symmetrical about the mean.

Table 2. Regression coefficients a and the coefficient of determination R^2 relating total variance VT to the mean, m , for each grade factor for farmers stock peanuts when using the USDA grade sample of 1800 g for foreign material and loose shelled kernels and a 500-g sample of pods for the remaining grade factors.

Grade factor	Regression coefficient a^a	Coefficient of determination
Foreign material	0.166	0.758
Loose shelled kernels	0.098	0.754
Sound mature kernels	0.115	0.822
Sound splits	0.211	0.865
Other kernels	0.170	0.884
Damaged kernels	0.363	0.791
Extra large kernels	0.335	0.923

$$^a VT = (am) - [(a/100) m^2].$$

For example, grade samples from a lot with $m = 65\%$ SMK will vary $65 \pm 3.2\%$ or between 61.8 and 68.2%, 95 times out of 100. The standard deviation of 3.2 is determined by taking the square root of 2.7 (using Eq. 3 and a in Table 2) and multiplying by 1.96. The factor 1.96 is associated with the 95% confidence limits.

The variance associated with the support price did not appear to vary with the mean and averaged about 160 across all lots studied. If a farmer had a lot with grade factors that supported a price of \$600.00, the support price as measured with the USDA 1800- and 500-g grade samples could vary \$600.00 \pm \$25.00 or between \$575.00 and \$625.00, 95% of the time.

Dowell (1992) used two grade sample sizes to partition the total variance into sampling and measurement error. The measurement error varied with grade factor since different types of equipment and human interactions were used with each grade factor. The ratio (expressed as a %) of VA to VT for %LSK, %FM, %SMK, %SS, %OK, and %DAM was 100, 39, 49, 100, 65, and 51%, respectively. The larger grade sample was double the official grade sample size and probably overloaded equipment and inspectors resulting in the larger-than-expected values of measurement error.

However, the measurement error measured by Dowell (1992) illustrates that one cannot assume that graders and equipment can do a perfect job of measuring each grade factor and care must be taken not to over load equipment with samples, keep equipment properly maintained, and properly train personnel. If these guidelines are followed, then the measurement error can be minimized.

Milled and In-Shell Peanuts

Grade factors associated with grading milled and in-shell peanuts were described by Davidson *et al.* (1982). No published information presently exists that describes the variability associated with measuring grade factors associated with grading milled and in-shell peanuts. However, the variance

relationships described by Eq. 3 and along with regression coefficients in Table 2 can be used as approximate estimates of variance until better data become available.

Error Reduction

The regression coefficients in Table 2 for Eq. 3 reflects the use of a 1800-g sample for %FM and %LSK and a 500-g sample for the remaining grade factors. Assuming measurement error is negligible, then doubling the 1800- and 500-g sample sizes should reduce the VS and VT associated with each grade factor and price support value in half. The variance relationship in Eq. 3 can be used to predict the variance expected for any number of USDA grade sample units, N:

$$VT \approx VS = (1/N)[am - (a/100)m^2] \quad \text{Eq. (4)}$$

A sampling unit is the official USDA grade sample size of 1800 g for %FM and %LSK and 500 g for the remaining grade factors. If measurement error becomes large compared to sampling error, then Eq. 4 will describe the sampling variance and not the total variance.

AFLATOXIN

In research, regulatory, and quality assurance activities, correct food safety decisions rely on accurate and precise measurements of the aflatoxin concentration in peanut lots. However, Whitaker *et al.* (1974, 1976, 1979), Campbell *et al.* (1986), and Park *et al.* (1991c) demonstrated the difficulty in estimating precisely the aflatoxin concentration in a large lot because of the variability associated with the aflatoxin test procedure. The testing procedure is a complicated process and generally consists of three steps: (a) a sample is taken from the lot, (b) the sample is comminuted to reduce particle size and a subsample is removed from the comminuted sample for analysis, and (c) the aflatoxin is extracted from the subsample and quantified.

Kratochvil and Taylor (1981) published general recommendations for sampling products for chemical analysis. Dickens and Whitaker (1982), Campbell *et al.* (1986), and Park and Pohland (1989) published reviews of accepted procedures for sampling and sample preparation methods for various agricultural commodities and listed various types of equipment used for sample preparation and sources of supply. Schuller *et al.* (1976), Nesheim (1979), and Park and Pohland (1986) published reviews of accepted analytical procedures to analyze various products for aflatoxin. Even when using accepted procedures, there is random variation associated with each step of the aflatoxin test procedure. Because of this variability, the true aflatoxin concentration in a peanut lot cannot be determined with 100% certainty by measuring the concentration in a sample taken from the lot.

The variability one might expect when inspecting a peanut lot for aflatoxin is illustrated in Table 3 by the 10-replicate sample test results taken from each of 20 aflatoxin contaminated raw-shelled peanut lots (Whitaker *et al.*, 1972). Each sample test result was made by comminuting a 5.45-kg sample

Table 3. Aflatoxin sample test results (ng/g) for 10-5.45-kg samples taken from 20 contaminated raw shelled peanut lots.^a

Lot No.	Sample										Avg	Variance
	1	2	3	4	5	6	7	8	9	10		
----- ng/g -----												
1	0	0	0	0	0	0	0	3	10	14	2.7	26.9
2	0	0	0	0	2	4	8	14	28	43	9.9	214.8
3	0	0	0	0	0	0	0	16	40	69	12.5	561.6
4	0	0	0	0	0	3	8	26	52	70	15.9	647.2
5	0	0	0	0	3	13	19	41	43	69	18.8	588.4
6	0	0	0	4	4	5	25	26	58	67	18.9	625.9
7	0	0	6	10	18	19	20	25	52	67	21.7	481.1
8	0	0	0	0	0	5	10	35	39	130	21.9	1663.9
9	0	0	3	12	12	12	25	63	103	103	24.2	1093.7
10	0	9	11	14	17	17	30	44	57	59	25.8	431.7
11	0	6	6	8	10	50	60	62	66	130	39.8	1732.8
12	3	3	9	12	41	42	57	70	80	126	44.3	1619.1
13	0	0	32	32	34	37	55	67	77	134	46.8	1563.3
14	0	3	5	19	32	49	87	91	127	168	58.1	3353.4
15	18	21	25	35	43	46	86	86	94	169	62.3	2229.4
16	11	19	23	38	54	90	96	108	132	140	71.1	2313.6
17	0	4	6	17	36	80	133	148	192	216	83.2	6871.7
18	5	12	56	66	70	92	98	132	141	164	83.6	2773.6
19	18	50	53	72	82	108	112	127	182	191	99.5	3168.8
20	29	37	41	71	95	117	168	174	183	197	111.2	4315.1

^aThe 10-sample test results from each lot have been ranked from low to high for ease of viewing.

of shelled peanut kernels in an USDA subsampling mill with a 3.2-mm screen (Dickens and Satterwhite, 1969; Dickens *et al.*, 1979), extracting aflatoxin from a 275-g subsample using the BF method or AOAC Method II (Helrich, 1990), and quantifying the aflatoxin densitometrically using thin layer chromatography (TLC) plates, (USDA, 1993).

The 10-sample test results from each lot in Table 3 are ranked from low to high to demonstrate several important characteristics about replicate aflatoxin test results taken from the same contaminated lot. First, the wide range among the replicate sample test results from the same lot is reflected in the large variances shown for each lot. The maximum sample result can be as much as five times the estimated 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 aflatoxin concentration increases, the variance among sample results increases. Thirdly, the distribution of the 10-sample results for each lot in Table 3 are not always symmetrical about the lot concentration (Whitaker *et al.*, 1972). The distribution of sample test

results are positively skewed, meaning that more than half of the sample results are below the 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 sample test results above and below the lot concentration in Table 3. If a single sample is tested from a contaminated lot, there is more than a 50% probability that the sample test result will be lower than the true lot concentration. The skewness is greater for small sample sizes and the distribution becomes more symmetrical as sample size increases (Remington and Schrok, 1970).

The variance shown for each lot in Table 3 is the sum of variances associated with each step of the sampling procedure. Total variance (VT) is equal to the sum of VS, subsampling variance (VSS) and VA:

$$VT = VS + VSS + VA \quad \text{Eq. (5)}$$

Estimates of the magnitude of these three variance components for several peanut products, several sample preparation techniques, and several type analytical methods are described below.

Sampling Variability

Studies by Whitaker *et al.* (1974, 1992, 1993) using raw shelled peanuts, screened in-shell peanuts, and farmers stock peanuts indicated that for small sample sizes, the sampling step is the largest source of variation in the testing procedure. Sampling variance is large because (a) aflatoxin is found only in a small percentage of the kernels in the lot and (b) the concentration in a single kernel may be extremely high (Whitaker and Wiser, 1969). A study by Whitaker *et al.* (1972) on raw shelled peanuts indicated that the percentage of contaminated kernels in a lot with an aflatoxin concentration of 20 parts per billion (ng/g) is 0.095% which is less than one contaminated kernel per 1000 kernels. The same studies also indicate that the percentage of aflatoxin contaminated kernels in a lot is a function of aflatoxin concentration and that the percentage contaminated kernels increases with lot concentration. However, these few contaminated kernels can have extremely high levels of aflatoxin. Cucullu *et al.* (1966, 1977) reported aflatoxin concentrations in excess of 1,000,000 ng/g for individual peanut kernels. A 5-kg sample of peanut kernels with a single kernel containing 10^6 ng of aflatoxin will have a sample concentration of 200 ng/g.

Because of the small percent of contaminated kernels and the high aflatoxin concentrations in individual kernels in a contaminated lot, variation among replicate sample test results are large. The sampling variance associated with raw shelled peanut kernels, VS_{pk} , screened in-shell peanuts, VS_{is} , and farmers stock peanuts, VS_{fs} , for a given sample size was estimated experimentally by Whitaker *et al.* (1974, 1992, 1993) and are shown in Eqs. 6, 7, and 8, respectively:

$$VS_{pk} = (49.3295M^{1.3955} - 1.9035M^{1.7867})/WS_{pk} \quad \text{Eq. (6)}$$

$$VS_{is} = 3.9539M/WS_{is} \quad \text{Eq. (7)}$$

$$VS_{fs} = (95.3565M^{0.9576})/WS_{fs} \quad \text{Eq. (8)}$$

where M is the aflatoxin concentration in the lot in ng/g, WS_{pk} is the mass of raw shelled peanut kernels in the sample in kg, WS_{is} is the mass of screened in-shell peanuts in the sample in kg, and WS_{fs} is the mass of farmers stock peanut pods in the sample in kg. From Eqs. 6, 7, and 8 one can see that the sampling variance increases with the aflatoxin concentration (M).

The sampling variances measured in the above studies assume that an unbiased sample is correctly selected from the lot. It is assumed that all kernels in the lot have an equal chance of being chosen during the sampling procedure and the variation measured in the above studies is random in nature.

Subsampling Variability

Once the sample has been taken from the lot, the sample must be prepared for aflatoxin extraction. Since it is not practical to extract the aflatoxin from a large sample, the aflatoxin is usually extracted from a small portion or subsample taken from a comminuted sample. It is essential that all kernels in the sample be comminuted in a suitable mill before the subsample is removed from the sample (Dickens and Whitaker, 1982; Park and Pohland, 1989). Removing a subsample of kernels from the sample before the comminution process eliminates the benefits associated with the larger size sample of kernels.

It is assumed that the distribution of contaminated particles in the comminuted sample is similar to the distribution of contaminated kernels found in the lot. As a result, there is also variability among replicate subsamples taken from the same comminuted sample. However, the subsampling variance should not be as large as the sampling variance due to the larger number of comminuted particles in the subsample than the number of kernels in the sample.

The subsampling variance has been measured for several types of mills. The subsampling variance for peanut kernels comminuted in the USDA subsampling mill, VSS_d (Whitaker *et al.*, 1974, 1992), and a Stephan vertical cutter mill, VSS_v (Dorner and Cole, 1993), is given below in Eqs. 9 and 10, respectively:

$$VSS_d = (0.0978M^{1.7867} - 0.0178M^{1.9339})/WSS \quad \text{Eq. (9)}$$

$$VSS_v = (0.01525M^{1.7920} - 0.003755M^{1.7573})/WSS \quad \text{Eq. (10)}$$

where M is the aflatoxin concentration in the sample in ng/g and WSS is the mass of comminuted peanut kernels in the subsample in kg. Regardless of the type mill used to comminute the sample, the subsampling variation increases with the aflatoxin concentration in the sample. The USDA subsampling mill uses a screen with 3.2 mm diameter holes to comminute peanut kernels. The USDA subsampling mill and the hole diameter in the screen are designed not to create a peanut paste. The Stephan mill comminutes the peanut kernels into a smaller particle size than the USDA subsampling mill and creates a peanut paste.

The effect of particle size reduction on the subsampling variance can be seen by comparing variances in Eqs. 9 and 10. For example, at a sample

concentration of 20 ng/g the subsampling variance associated with a 250-g subsample using the USDA and Stephan mills is 59.2 and 10.2, respectively. The coefficient of variation (CV) is 38.5 and 16.0%, respectively. The subsampling variance for peanut kernels at 20 ng/g is reduced by a factor of 6, due mostly to a reduction in particle size.

The subsampling variance is much lower than sampling variance and is also a function of the aflatoxin concentration in the sample. For a given subsample size, the subsampling variance is reduced by increasing the degree of comminution or increasing the number of particles per unit mass.

Analytical Variability

Once the subsample is removed from the comminuted sample, the aflatoxin is usually extracted by official methods (Schuller *et al.*, 1976; Nesheim, 1979; Park and Pohland, 1986). These methods usually involve several steps such as solvent extraction, centrifugations, drying, dilutions, and quantification. As a result, there can be considerable variation among replicated analyses on the same subsample extract. Whitaker *et al.* (1974) and Whitaker and Dickens (1981) determined the analytical variance, VA_{bf} , associated with the BF method or the AOAC Method II extraction procedure along with TLC and densitometric quantification techniques to measure aflatoxin in peanuts (Helrich, 1990). The analytical variance, VA_{bf} , associated with the BF method is:

$$VA_{bf} = 0.0637M^{1.9339}/N_{bf} \quad \text{Eq. (11)}$$

where M is the aflatoxin concentration in the subsample in ng/g, and N_{bf} is the number of aliquots quantified by TLC methods. For example, at 20 ng/g, the CV associated with the BF method is 22.8%. Studies by Whitaker and Dickens (1981) on the BF method indicate that the thin layer chromatography quantification step is the major source of variability in the analytical process associated with analyzing peanuts for aflatoxin.

If extraction and cleanup contribute only a small portion of the total analytical variance then the immunoassay and high performance liquid chromatography (HPLC) type analytical methods should have lower variances than methods that use TLC quantification techniques. Hagler and Whitaker (1991) and Dorner and Cole (1988) independently measured the analytical variance associated with HPLC type methods. Even though Hagler and Dorner used slightly different extraction and cleanup procedures (Dorner and Cole, 1988; Wilson and Romer, 1991), both obtained almost identical results. The relationship between variance and aflatoxin concentration of Hagler's study for HPLC are given below:

$$VA_h = 0.004828M^{1.7518}/N_h \quad \text{Eq. (12)}$$

where M is the aflatoxin concentration in the subsample and N_h is the number of aliquots quantified by the HPLC procedure. At 20 ng/g, the CV associated with the HPLC method is 4.8%. A CV of 4.8% associated with HPLC is much lower than the 22.8% associated with the BF and CB methods using TLC quantification techniques.

Immunoassay techniques have been applied to the measurement of aflatoxin in several commodities such as peanuts, corn, and cottonseed only recently. Food and feed industries along with regulatory agencies are studying the variability associated with immunoassay type analytical methods (Whitaker, unpubl. data, 1991; Park *et al.*, 1991a,b,c). The variability one might expect using an immunoassay-type analytical methods is given below:

$$VA_i = 0.01327M^{1.5651}/N_i \quad \text{Eq. (13)}$$

where M is the aflatoxin concentration in the subsample and N_i is the number of aliquots quantified by the immunoassay procedure. Equation 13 specifically reflects the Aflatest method and a pooling of variance data from corn, cottonseed, and peanuts. At 20 ng/g, the CV computed from Equation 13 is 6.0%. The variability associated with immunoassay type methods appears to be less than TLC methods and more than HPLC methods.

All of the analytical variance information described above reflects results from single laboratories and do not reflect among laboratory variances. As a result, some laboratories may have higher or lower variances than those reported in Eqs. 11, 12, and 13.

Error Reduction

Because of the large variability associated with aflatoxin test procedures, it is difficult to estimate with a high degree of confidence the true aflatoxin concentration of a lot. The only way to achieve a more precise estimate of the lot concentration is to reduce the total variance or the individual variance components associated with each step of the test procedure. The sampling variance can be reduced by increasing the size of the sample. The subsampling variance can be reduced either by increasing the size of the subsample and/or by increasing the degree of comminution (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 more precise quantification methods (i.e., using HPLC instead of TLC). If one or more of these variance components are reduced, then the total variance associated with the test procedure can be reduced.

Sampling variance in Eqs. 6, 7, and 8 have a sample size term; subsampling variance in Eqs. 9 and 10 have a subsample size term; and analytical variance in Eqs. 11, 12, and 13 have terms for the number of aliquots quantified by the analytical method. The effect of increasing sample size, subsample size, or the number of aliquots on reducing variance can be determined from these equations. The sampling and subsampling variances, described by Eqs. 6 to 10, specify sample and subsample size in mass rather than number of kernels in the sample and number of particles in the subsample. The number of kernels in the sample and the number of particles in the subsample is statistically the better way to specify sample and subsample size. However, mass is used in the previous variance equations because mass is directly correlated with the number of kernels or particles for a given commodity and because mass is a more convenient measurement than number of particles.

Variance reduction is inversely proportional to sample size. For example, if the sample size of any commodity is doubled, then the sampling variance is decreased by half (Walpole, 1974). Likewise if the subsample size and number of aliquots quantified is doubled, the subsampling and analytical variance components are each decreased by half.

Substituting the appropriate variance equation for each step of the sampling procedure into Eq. 5 for total variance, one can determine if the expected total variance among sample test results is acceptable and determine the most efficient method to reduce variation. For example, the expected total variance associated with testing a raw shelled peanut lot with 20 ng/g aflatoxin when using a 5.45-kg sample, a USDA subsampling mill, an 1100-g subsample, and TLC can be estimated by summing Eqs. 6, 9, and 11 as follows:

$$VT = 575.1 + 13.5 + 20.9 = 609.5 \quad \text{Eq. (14)}$$

The variance, standard deviation, and CV associated with the test procedure described above is 609.5, 24.7, and 123%, respectively. The sampling, subsampling, and analytical variances accounts for 94.4, 2.2, and 3.4% of the total variance, respectively. The major variance component is the sampling variance associated with the 5.45-kg sample and accounts for 94.4% of the total variation. It appears that the best use of resources to reduce the total variance would be to increase sample size. Increasing the sample size by a factor of four from 5.45 to 21.8 kg decreases the sampling variance term in Equation 14 by a factor of four from 575.1 to 143.8. The total variance with the 21.8-kg sample now becomes:

$$VT = 143.8 + 13.5 + 20.9 = 178.2 \quad \text{Eq. (15)}$$

The variance, standard deviation, and CV associated with the test procedure has been reduced to 178.2, 13.3, and 66.7%, respectively.

The range of sample test results associated with the two test procedures described by Equations 14 and 15 when sampling a lot of raw shelled peanuts with an aflatoxin concentration of 20 ng/g can be estimated from the standard deviation SD, which can be determined by taking the square root of the total variance in Equations 14 and 15. Approximately 95% of all test results will fall between a low of $(M - 1.96SD)$ and a high of $(M + 1.96SD)$. The two expressions are only valid for a normal distribution where sample test results are symmetrical about the mean. The distribution of sample test results are usually skewed, but approach a symmetrical distribution as sample size becomes large. The expected range of sample test results for the test procedure using a 5.45-kg sample (Eq. 14) is $20 \pm 1.96 \times 24.7$, or 0 to 68.4 ng/g. The expected range of sample test results for the test procedure using the 21.8-kg sample (Eq. 15) is $20 \pm 1.96 \times 13.2$, or 0 to 46.2 ng/g.

As indicated above, there are methods other than increasing sample size to reduce the total variance associated with testing peanuts for aflatoxin. Different costs are associated with each method and careful study is required to determine the testing procedure that will provide the lowest variance for

a given cost. The optimum balance in sample size, degree of comminution, subsample size, number and type of analysis will vary with the costs involved with each step of the testing procedure. In general, the costs of properly designed aflatoxin sampling procedures will increase as the total variance is reduced.

Designing Aflatoxin Sampling Plans

An aflatoxin sampling plan is defined by (a) the aflatoxin test procedure and (b) the definition of good and bad lots according to the lot aflatoxin concentration. Often the national guideline, M_c , is used to define the difference between acceptable and unacceptable lots. However, any sample acceptance level, X_c , can be used to define acceptable and unacceptable lots. While X_c is usually equal to the national guideline, X_c can be any concentration greater than or less than the guideline. A lot is termed bad when the sample test result, X , is above some predefined sample acceptance level, X_c . The lot is termed good when X is less than or equal to X_c .

Because of the large variability associated with the aflatoxin test procedure, two types of mistakes or risks are associated with any aflatoxin sampling plan. First, good lots (lots with a concentration less than or equal to the guideline) may test bad and be rejected by the sampling plan. The chance of making this type of mistake is often called the processor's risk since these lots will be rejected at an unnecessary cost to the processor. Secondly, bad lots (lots with a concentration greater than the guideline) may test good and be accepted by the sampling plan. The chances of making this type of mistake is often called the consumer's risk since contaminated lots have a potential for making their way into the food chain. In order to maintain an effective quality control program, the above risks associated with a sampling plan must be evaluated. Based upon the evaluation of the costs and benefits (benefits refers to removal of aflatoxin contaminated lots) effective aflatoxin sampling plans can then be designed.

For a given sampling design, lots with an aflatoxin concentration M will be accepted with a certain probability $P(M)$ by the sampling plan. A plot of $P(M)$ versus M is called an operating characteristic (OC) curve. Figure 4 depicts the general shape of an OC curve. As M approaches 0, $P(M)$ approaches 1 (100%), and as M becomes large, $P(M)$ approaches zero. The shape of the OC curve is uniquely defined for a particular sampling plan with designated values of sample size, degree of comminution, subsample size, number of analyses and the sample acceptance level X_c .

For a given sampling plan, the OC curve indicates the magnitude of the processor's and consumer's risks. In Fig. 4, the area under the OC curve for $M > M_c$ represents the consumer's risk (bad lots accepted) while the area above the OC curve for $M > M_c$ represents the processor's risk (good lots rejected) for a particular sampling plan. Because the shape of the OC curve is uniquely defined by the sample size, type mill used to comminute the sample, subsample size, the number of analyses and the sample acceptance level X_c , these parameters can be used to reduce the processor's and consumer's risks associated with a sampling plan.

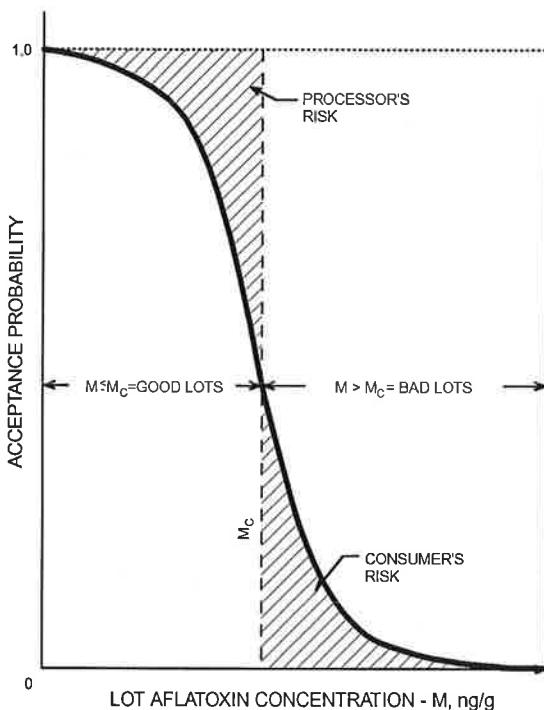


Fig. 4. Typical operating characteristic curve showing processor's and consumer's risks.

The effect of increasing sample size on the shape of the OC curve when sampling raw shelled peanut lots for aflatoxin is shown in Fig. 5 where the sample acceptance level is equal to the guideline of 15 ng/g. As sample size increases from 3 to 12 to 48 kg, the slope of the OC curve about M_c also increases forcing the two areas associated with each risk to decrease. As a result, increasing sample size decreases both the processor's and consumer's risk. The same effect (but to a lesser extent) can be obtained by increasing either the degree of sample comminution, increasing subsample size, or increasing the number of analyses. In effect, reducing the variability associated with each step of the test procedure will reduce both the processor's risk and the consumer's risk.

The effect of changing the sample acceptance level, X_c , on the two risks when sampling raw shelled peanut lots for aflatoxin is shown in Fig. 6. If the guideline is assumed to be 15 ng/g, then changing X_c to a value less than 15 ng/g shifts the OC curve to the left. For example, the sampling plan where $X_c = 10$ ng/g has a much lower consumer's risk and a much higher processor's risk than the sampling plan where $X_c = 15$ ng/g. If X_c becomes larger than 15 ng/g, the OC curve shifts to the right. The sampling plan where $X_c = 20$ ng/g has a much lower processor's risk and a much higher consumer's risk than the sampling plan where $X_c = 15$ ng/g. As a result, only one of the two risks can be reduced by changing X_c relative to the guideline, because

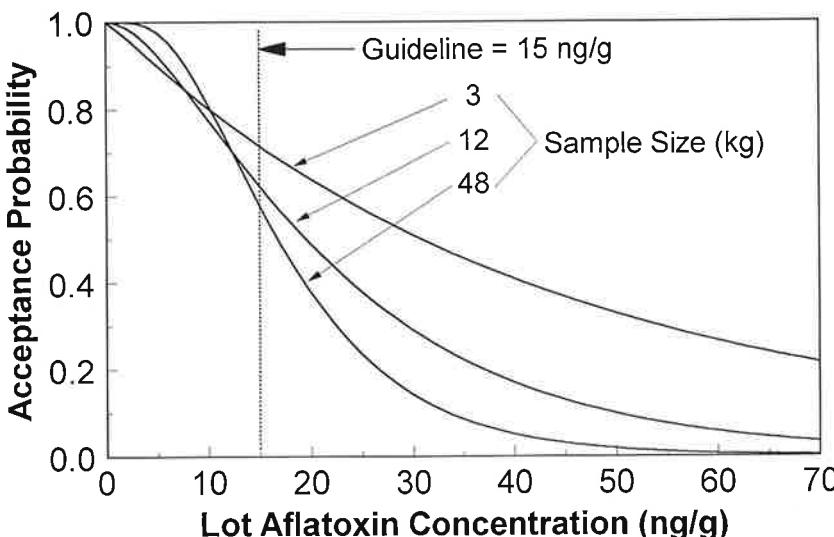


Fig. 5. Operating characteristic curves for 3-, 12-, and 48-kg samples of raw shelled peanuts where the sample acceptance level equals the guideline of 15 ng/g.

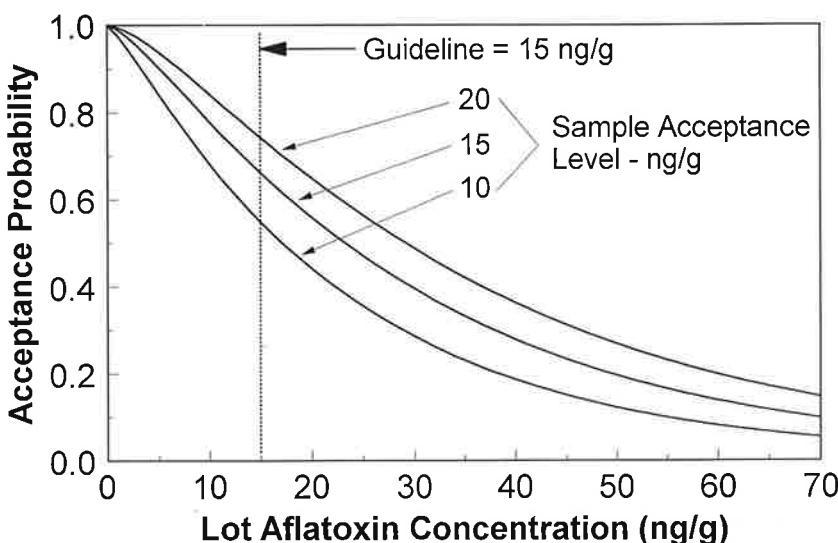


Fig. 6. Operating characteristic curves for a 6-kg sample of raw shelled peanuts and a 15-kg/g guideline when using 10-, 15-, and 20-ng/g sample acceptance levels.

reducing one risk will automatically increase the other risk. The above discussion about the effect of X_c on the processor's risk and the consumer's risk assume the sample size, sample preparation techniques, and analytical methods are the same.

Statistical Evaluation Method

Whitaker (1977) developed methods to evaluate aflatoxin sampling designs and compute the OC curve or the acceptance probabilities associated with a given aflatoxin sampling plan. Whitaker and Wiser (1969) used the negative binomial function to describe the distribution of sample test results (shown in Table 3) for raw shelled peanuts (Whitaker *et al.*, 1974), screened in-shell peanuts (Whitaker *et al.*, 1992), and farmers stock peanuts (Whitaker *et al.*, 1993, 1994a,b). The evaluation method has been used extensively to design the USDA-Peanut Administrative Committee's (PAC) aflatoxin sampling plan used for raw shelled peanuts (Whitaker and Dickens, 1979).

Since 1975 the PAC aflatoxin sampling plan has been a modified sequential sampling plan where one, two, or three 21.8-kg samples are used to accept or reject a lot of raw-shelled peanuts. Three 21.8-kg samples are removed from the lot and each sample is comminuted in an USDA subsampling mill. A 1100-g subsample of comminuted peanuts is removed from each sample. The aflatoxin in each subsample is extracted using a slurry modification of AOAC Method II. Two aliquots are taken from each subsample extract and quantified using TLC methods (USDA, 1993). The aflatoxin in the two aliquots are averaged. If the aflatoxin test result in the first sample is 8 ng/g or less, then the lot is accepted and no additional samples are tested. If the aflatoxin test result in the first sample is greater than 45 ng/g, then the lot is rejected and no additional samples are tested. If the aflatoxin test result from the first sample is between 8 and 45 ng/g, then the second sample is tested for aflatoxin and results are averaged with the first sample. If the average of the two sample tests results is 12 ng/g or less, then the lot is accepted and no additional samples are tested. If the average of the two sample test results is greater than 23 ng/g, then the lot is rejected and no additional samples are tested. If the average of the two sample test results is between 12 and 23 ng/g, then the third sample is tested for aflatoxin and all three sample test results are averaged. If the average of the three sample test results is 15 ng/g or less, then the lot is accepted. If the average of the three sample test results is greater than 15 ng/g, then the lot is rejected.

The advantage of a sequential type sampling plan is that most good lots, with little or no aflatoxin, will be accepted with one sample and most bad lots, with high aflatoxin concentrations, will be rejected with one sample. However, lots with concentrations near the final accept level of 15 ng/g will require the use of two or three samples to accept or reject the lot. The sampling plan gives the protection of three 21.8-kg samples (or 65.4 kg) while using much less than 65.4 kg of sample per lot on the average across all lots sampled in a crop season.

While the sample size and the sequential structure of the USDA sampling plan has remained the same since 1975, three different sample acceptance levels have been used. From 1975 to 1987 the final acceptance level was 25 ng/g, during 1988 to 1989 the final acceptance level was 20 ng/g, and since 1990 the final acceptance level is 15 ng/g (Table 4).

Using the negative binomial equation along with variance estimates for a 21.8-kg sample (Eq. 6), a 1100-g subsample from the USDA subsampling

Table 4. Accept and reject concentrations used in the USDA, Peanut Administrative Committee's aflatoxin sampling plan for shelled peanuts since 1975.

Year	Sample no.					
	1		1+2 ^a		1+2+3 ^b	
	Accept if \leq	Reject if $>$	Accept if \leq	Reject if $>$	Accept if \leq	Reject if \geq
----- ng/g -----						
1975-1987	16	75 ^c	22	38	25	26
1988-1989	12	60	17	30	20	21
1990-	8	45	12	23	15	16

^aAverage of samples one and two.

^bAverage of samples one, two, and three.

^cIf sample test result is between the accept and reject concentration, the next sample is analyzed for aflatoxin.

mill (Eq. 9) and the TLC quantification (Eq. 11), three OC curves describing the USDA sampling plans used since 1975 are shown in Fig. 7. By reducing the final sample acceptance levels, the PAC sampling plan rejects more lots at all aflatoxin concentrations.

Number of Lots Accepted and Rejected

The performance of a sampling plan when used to inspect a peanut lot with

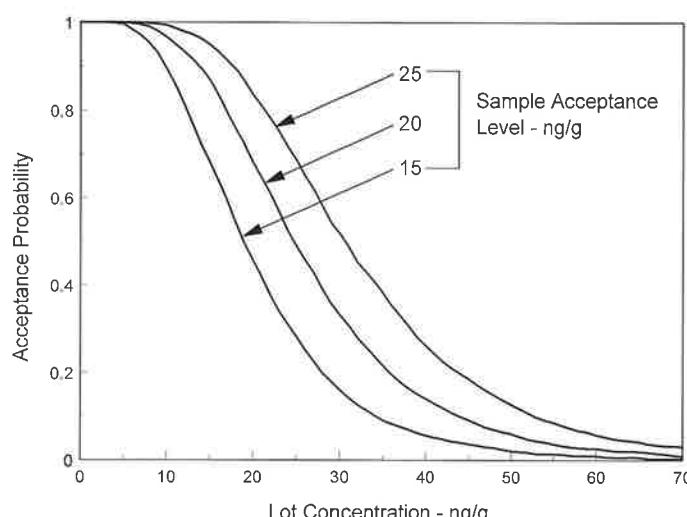


Fig. 7. Operating characteristic curves describing the aflatoxin sampling plans used since 1975 for raw shelled peanuts marketed in the U.S. The sample acceptance level of 25 ng/g was used from 1975 through 1987, 20 ng/g was used from 1988 through 1989, and 15 ng/g was used from 1990 to present.

a given aflatoxin concentration is described by the OC curve. The performance of a sampling plan for inspecting a collection of lots, such as lots marketed over a crop year, requires the additional specification of a lot distribution. A lot distribution describes the frequency distribution of lot aflatoxin concentrations among a collection of lots.

A lot distribution, shown in Table 5, was constructed from aflatoxin test results on 311,000 raw shelled peanut lots inspected over a 10-year period from 1976 to 1985. The average amount of aflatoxin among all lots in the distribution shown in Table 5 is 5.3 ng/g. The estimated lot distribution describes an average crop year in terms of aflatoxin contamination. Whitaker and Dickens (1979) showed how to convert the accept probabilities given by the OC curve into number of lots accepted and rejected by coupling the OC curve and lot distribution together. For example, the lot distribution shown in Table 5 is used to convert the accept probabilities associated with the three PAC sampling plans used since 1975, OC curves shown in Fig. 7, to number of lots accepted and rejected and the amount of aflatoxin removed from the food chain.

The effect of lowering the PAC final acceptance level from 25 to 20 to 15 ppb on the number of lots accepted, the number of lots rejected, and the amount of aflatoxin removed from all lots inspected is shown in Table 6 when

Table 5. Cumulative distribution among lot aflatoxin concentrations for raw shelled peanuts.^a

Lot aflatoxin concentration ^b ng/g	Cumulative frequency distribution %
0	50.00
1	60.00
2	65.00
3	69.00
4	72.33
5	75.00
6	77.33
7	79.33
8	81.03
9	82.57
10	84.00
15	89.60
20	93.10
25	95.10
30	96.40
40	97.60
50	98.33
70	99.16
100	99.75

^a Lot distribution constructed from USDA aflatoxin test results on 311,000 lots inspected from 1976 to 1985.

^b The average amount of aflatoxin among all lots in the distribution is 5.3 ng/g.

Table 6. Number of lots accepted and rejected and the average aflatoxin concentration among accepted lots when inspecting 30,000 raw shelled peanut lots for aflatoxin using the USDA aflatoxin sampling plan with different final sample acceptance levels.^a

Final acceptance level ng/g	Lots accepted No.	Lots rejected No.	Avg aflatoxin among accepted lots ^b ng/g
25	28,589	1,411	3.4
20	28,061	1,939	3.1
15	27,150	2,850	2.6

^a Inspected a total of 30,000 lots.

^b Average aflatoxin concentration among 30,000 lots was 5.3 ng/g.

inspecting the lot distribution described in Table 5. By decreasing the sample acceptance level from 25 to 15 ng/g, the percent lots accepted decreased from 95.3 to 90.5% of all lots tested; the percent lots rejected increases from 4.7 to 9.5% of all lots tested; and the average aflatoxin concentration in the accepted lots decreases from 3.4 to 2.6 ng/g.

Lowering the final acceptance level from 25 to 15 ng/g has had the desired effect of removing more contaminated lots from the food chain. However, the cost to the peanut industry has increased as the total number of lots rejected has approximately doubled from 1411 to 2850.

Whitaker *et al.* (1994b) have made similar evaluations of the performance of sampling plans for farmers stock peanuts. The effect of sample size and sample acceptance levels on the number of farmers stock lots classified segregation I and segregation III and the amount of aflatoxin removed from lots before storage have been evaluated.

CONCLUSIONS

Because of the variability associated with sampling peanuts for grade factors and aflatoxin, it is difficult to determine with 100% certainty the true characteristics of a peanut lot. Even when the sample is correctly selected, there will be variability associated with the testing procedure. For grade factors, the total variance is the sum of sampling and measurement variances. For aflatoxin, the total testing variance is the sum of sampling, subsampling, and measurement variances. For small sample sizes, sampling is usually the largest source of variability. For aflatoxin sampling plans, increasing the sample size, the degree of sample comminution, subsample size, or the number of analyses, increases the precision of the test procedure and also decreases both the processor's and consumer's risk associated with a sampling plan.

Decreasing the sample acceptance level below the guideline increases the processor's risk, but decreases the consumer's risk. Conversely, increasing the sample acceptance level above the guideline reduces the processor's risk,

but increases the consumer's risk. The entire sample should be comminuted before a subsample is removed from the sample. Increasing the degree of comminution and increasing subsample size reduces the subsampling variability. Care should also be taken in drawing the sample from the lot. The sample should be a composite of many small incremental portions taken from different locations throughout the lot.

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