Design and Analysis of Sampling Plans to Estimate Aflatoxin Concentrations in Shelled Peanuts[1]

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Abstract

Methodology for use in the design and evaluation of sampling plans to estimate aflatoxin concentrations in lots of shelled peanuts is presented. Use of the operating characteristic curve for comparing and evaluating processor and consumer risks related to various sampling plans and application of the negative binomial distribution to estimate probabilities associated with sampling lots of shelled peanuts for aflatoxin concentration are discussed. Operating characteristic curves are developed for two different single-sample plans, an attribute multiple sample plan, and the plan presently used by the peanut industry to estimate aflatoxin concentrations in commercial lots of shelled peanuts. An estimated prior distribution of lots according to aflatoxin concentration is used to predict, among others, such values as the per cent of all lots tested that will be accepted by the sampling plans and the average aflatoxin concentration in the accepted lots. All four of the sampling plans described in the paper are compared on the basis of values such as these. Other factors to be considered in the critical evaluation and selection of sampling plans for estimating aflatoxin concentrations in commercial lots of shelled peanuts are discussed.

Introduction

Aflatoxin is a toxic material produced in peanuts by the fungus *Aspergillus flavus* (1). As a precautionary measure, all shelled peanuts are tested for aflatoxin prior to processing for food use. Estimates of the average level of aflatoxin are based upon analyses of samples taken from the lots. A lot of shelled peanuts may vary in size up to 100,000 lb. To facilitate an adequate quality control and consumer protection program within the peanut industry, it is desirable to design a sampling plan that will provide a high level of protection for the consumer with reasonable assurance to the processor that lots of good peanuts will not be rejected by the testing program.

Because aflatoxin is often highly concentrated in a very small percentage of the kernels, variation among sample means is large and determination of the average concentration in a lot is exceedingly difficult. Samples from a good lot may indicate that the lot is bad (processors' risk) and at other times samples from a bad lot may indicate that the lot is good (consumers' risk). Since the average aflatoxin concentration cannot be determined exactly from samples, it is advantageous to compute the confidence levels associated with these determinations.

In this paper the design and evaluation of sampling plans for the determination of aflatoxin contamination are discussed, and a method of evaluating a testing program to predict the number of good lots rejected and the number of bad lots accepted is presented. Four sampling plans, including one used by the peanut industry for the 1969 crop, are analyzed and discussed.

Method of Analyzing Sampling Plans

As a consequence of a sampling plan, a lot of shelled peanuts is judged acceptable or unacceptable depending upon analyses of samples drawn from the lot. For a given lot, the consumers' and processors' risks are functions of the sample size N and the definition of good and bad sample quality. A sample may be termed bad when its sample mean $\overline{X}$ is above some predefined success level $\overline{X}_s$ and good when $\overline{X} \leqq X_s$. Lots with an average concentration of aflatoxin, M, will be accepted as good with a certain probability $P(M) = (\overline{X} \leqq \overline{X}_s|M)$.

A plot of the probability P(M) versus M is called an operating characteristic (OC) curve. Figure 1 depicts the general shape of an OC-curve. As M approaches zero P(M) approaches 1, 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 N and $\overline{X}_s$ and the probability distribution of $\overline{X}$.

For a given sampling plan, the OC curve gives an indication of the magnitudes of the consumers' and processors' risks. When M_c is defined as the maximum average concentration of aflatoxin acceptable, lots with $M > M_c$ are bad and lots with $M \leqq M_c$ are good. In Figure 1, the area beneath the OC curve for $M > M_c$ is indicative of the consumers' risk while the area above the OC curve for $M \leqq M_c$ is indicative of the processors' risk for a particular sampling plan.

The areas above and below the OC curve, which are related to the consumers' and processors' risks, can

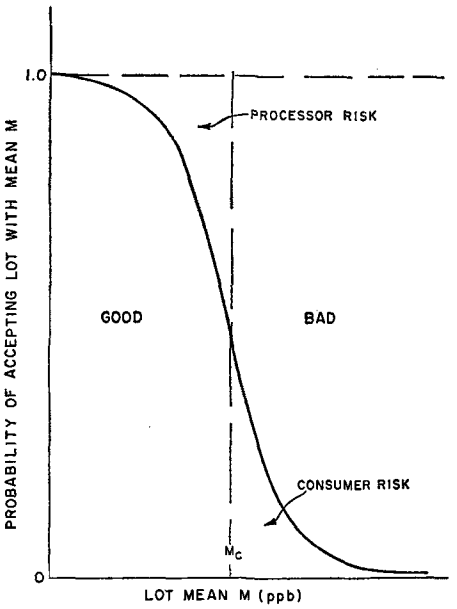

FIG. 1. Typical operating characteristic curve for evaluating sampling plans.

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be quantified if the prior distribution of all lot means is estimated. The prior distribution is estimated from the frequency distribution of lot means computed from previous observations. The total number of lots having an average aflatoxin concentration M is $L \cdot f(M)$ where L is the total number of lots and f(M) is the percentage of L lots with M as indicated by Table I. For a given sampling plan, the total number of lots accepted may be computed with the following equation where P(M) is obtained from the OC curve.

$$L_a = \sum_{M=0}^{\infty} L \cdot f(M) \cdot P(M). \quad [1]$$

The number of good lots accepted is

$$GL_a = \sum_{M=0}^{M_c} L \cdot f(M) \cdot P(M), \quad [2]$$

while the number of bad lots accepted is

$$BL_a = \sum_{M=M_c+\Delta}^{\infty} L \cdot f(M) \cdot P(M), \quad [3]$$

where Δ is the next measurable increment above M_c. The number of good lots rejected is

$$GL_r = \left[\sum_{M=0}^{M_c} L \cdot f(M) \right] - GL_a. \quad [4]$$

The number of bad lots accepted is indicative of the consumers' risk while the number of good lots rejected is indicative of the processors' risk associated with a given sampling plan. The average amount of aflatoxin in those lots accepted by a given sampling plan is

$$A = \sum_{M=0}^{M=\infty} M \cdot L \cdot f(M) \cdot P(M)/L_a. \quad [5]$$

Theoretical Model

The first step in the procedure to compute the OC curve for a given sampling plan is to determine the distribution of sample means as a function of sample size N and sample quality $\overline{X}$. In a previous paper (2), the authors described the use of the negative binomial distribution to describe properties of samples drawn from lots of shelled peanuts. The extent to which the negative binomial distribution satisfactorily matches the frequency distribution of kernels from a lot according to aflatoxin concentration has not been determined; but it does have desirable properties such as allowing high probabilities of zero amounts with low probabilities of very large amounts. In this paper, it is assumed that the negative binomial distribution matches the frequency distribution of kernels according to aflatoxin contamination within typical lots of contaminated shelled peanuts. Two parameters used to define the distribution are mean concentration M in the lot, and the proportion of peanuts having zero aflatoxin concentration F(O). By assigning values to these parameters for a population, it is possible to determine the distribution of sample means $\overline{X}$ for various sample sizes N as shown in the paper referred to above (2).

TABLE I

Estimated Prior Distribution of 20,000 Lots of Shelled Peanuts According to Lot Means M and Corresponding Probabilities for Accepting Lots in One Trial when N = 18,000 and $\overline{X} \leq 3$ ppb[a]

Lot M ppb	% of Lots	No. of Lots	Prob. of Accepting Lot[b]
0–2	55.0	11,000	0.990
2–4	9.0	1,800	0.610
4–6	6.0	1,200	0.367
6–10	5.0	1,000	0.225
10–15	5.0	1,000	0.125
15–20	4.5	900	0.087
20–30	4.0	800	0.058
30–40	3.5	700	0.030
40–60	2.5	500	0.020
60–80	2.0	400	0.015
80–100	1.0	200	0.010
Over 100	2.5	500	0.000

[a] The distribution is estimated from data on the 1967 crop of peanuts which were supplied by the Fruit and Vegetable Division, CMS, USDA.
[b] Probabilities are for the average of the specified range of M.

The confidence level associated with a given sampling plan is inversely proportional to the parameter F(O). Since F(O) will vary among lots, it is desirable to designate an F(O) value that is high enough to provide a reasonable margin of safety for the consumer in the calculated confidence levels. Cucullu (3) indicates that F(O) can be as high as 99.8% for shelled peanuts. Also, Dickens (unpublished data) indicates that the average amount of aflatoxin in contaminated peanut kernels may be as high as 50,000 ppb. If only 0.1% were contaminated and these kernels contained 50,000 ppb, the average concentration in a lot could not exceed 50 ppb. Therefore, F(O) = 99.9% indicates higher consumer and processor risks than may normally occur in practice and is used in this paper to provide adequate protection against acceptance of lots with high levels of contamination.

Basic assumptions underlying use of the theoretical model are: (a) the distribution of aflatoxin-contaminated kernels is described by the negative binomial distribution, (b) the sample is drawn in a random manner, (c) there are no subsampling errors, (d) there are no sample analysis errors, and (e) the value assigned to F(O) is appropriate.

Using the negative binomial distribution for F(O) = 99.9% the cumulative distribution of sample means was computed for various values of M and N as presented in Table II. The Table is generalized so that the probabilities apply to a wide range of means M. When M is specified the sample means may be computed and the associated probabilities for $\overline{X}$ and N can be read from the Table. For example, the probability of drawing a sample of 25,000 kernels (N) with a mean $\overline{X} \leq 3$ ppb (0.1 M) from a lot with a true mean M = 30 ppb is 0.019.

Development of Sampling Plans and OC Curves

Single Samples: Plans 1 and 2

Perhaps the simplest type of sampling plan is to estimate the M of a lot based on the $\overline{X}$ for a single sample of size N. The value of $\overline{X}$ for acceptance $\overline{X}_s$ and N are chosen to minimize the consumers' and processors' risks.

A 95% probability of rejecting a lot with M = 30 ppb is provided by letting $\overline{X} = \overline{X}_s$ in Table II and selecting N and $\overline{X}_s$ values which are associated with probabilities of approximately 0.05. Several combinations of N and $\overline{X}_s$ exist that give P(30) = 0.05. For example, N = 18,000 kernels and $\overline{X}_s = 3$ ppb gives P(30) = 0.047, while N = 100,000 kernels and $\overline{X}_s = 15$ ppb gives P(30) = 0.054. To find all the points on the OC curve for a designated N and $\overline{X}_s$, assign a range of values to M and plot the corresponding probabilities of accepting the lot versus the assigned values of M. OC curves for $\overline{X}_s = 18$ ppb, N = 12,000 kernels (Plan 1) and for $\overline{X}_s = 18$ ppb, N = 35,000 kernels (Plan 2) are plotted in Figure 2.

Multiple Samples: Plans 3 and 4

There are several types of multiple-sampling plans; only the attribute type is discussed here. With attri-

TABLE II

Probability of Obtaining a Sample Mean, $\bar{X}$, or Less From a Lot of Shelled Peanuts With Mean M Computed From the Negative Binomial Distribution

Sample Mean $\bar{X}$, ppb	Sample size N in thousands of kernels									
	5	7	9	12	18	25	35	50	70	100
0.1 M	0.32	0.23	0.17	0.11	0.047	0.019	0.006	0.001	0.000	0.0000
0.2 M	0.41	0.32	0.26	0.20	0.12	0.065	0.030	0.010	0.003	0.0000
0.3 M	0.47	0.40	0.34	0.27	0.19	0.13	0.075	0.036	0.014	0.0038
0.4 M	0.52	0.46	0.40	0.34	0.26	0.20	0.14	0.083	0.044	0.018
0.5 M	0.56	0.50	0.46	0.41	0.33	0.27	0.21	0.15	0.097	0.054
0.6 M	0.60	0.55	0.51	0.46	0.40	0.34	0.29	0.23	0.17	0.12
0.7 M	0.63	0.59	0.55	0.51	0.46	0.41	0.36	0.31	0.26	0.21
0.8 M	0.66	0.62	0.59	0.56	0.51	0.48	0.44	0.40	0.36	0.32
0.9 M	0.68	0.65	0.62	0.60	0.57	0.54	0.51	0.49	0.46	0.43
1.0 M	0.70	0.68	0.66	0.64	0.61	0.60	0.58	0.57	0.56	0.54
1.2 M	0.74	0.72	0.71	0.70	0.69	0.69	0.70	0.70	0.72	0.74
1.6 M	0.80	0.79	0.79	0.80	0.81	0.83	0.85	0.88	0.91	0.94
2.0 M	0.84	0.84	0.85	0.86	0.88	0.91	0.92	0.96	0.98	0.99
4.0 M	0.94	0.96	0.97	0.98	0.99	1.00	1.00	1.00	1.00	1.00

bute sampling, several samples are drawn from the lot and each $\bar{X}$ is given some predefined test. Depending upon the value of $\bar{X}$ the sample is classed into one of two categories, success if $\bar{X} \leqq \bar{X}_s$, or failure if $\bar{X} > \bar{X}_s$. The magnitude of the $\bar{X}$ carries no significance other than to class the sample into one of the above categories. Depending upon the number of successes S out of a total of T trials, the lot is accepted or rejected. The probability of obtaining at least S successes out of T trials is computed by the cumulative binomial distribution. Therefore, the probability P(S) of accepting a lot is given in Equation 6.

$$P(S) = \sum_{s=S}^{T} \binom{T}{s} p^s q^{(T-s)} \qquad [6]$$

where p is the probability of obtaining one success from one trial, q = 1–p, and $\binom{T}{s}$ is an expression for the binomial coefficients. The value of p is determined from the distribution of sample means given by Table II.

Probabilities associated with attribute multiple-sampling plans can be computed using Table II and cumulative binomial tables (4). For example, when S = 1, T = 1, N = 12,000, $\bar{X} \leqq 4$ ppb, and M = 20 ppb; Table II shows that p = 0.20. Then, from cumulative binomial tables the probability P of obtaining two successes out of two trials for p = 0.20 is 0.04. The probability P of accepting a lot with mean M for some values of N, $\bar{X}_s$, S and T is presented in Table III.

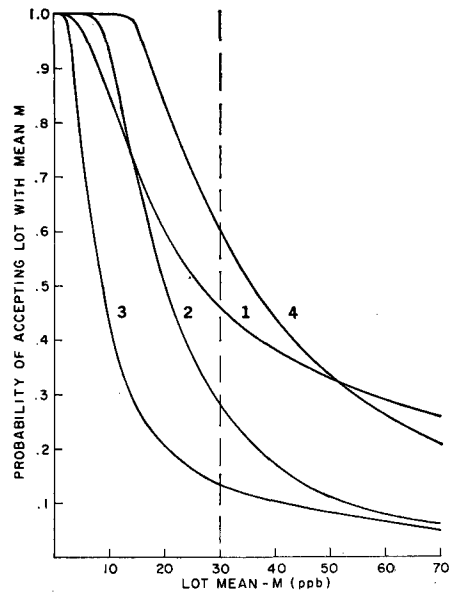

FIG. 2. Operating characteristic curves for sampling plans 1–4.

Plan 3

An example of an attribute multiple-sampling plan requiring a sample size N = 18,000 kernels and at least one success from three trials follows: Step 1: test a sample and accept the lot if $X \leqq 3$ ppb. Step 2: if $\bar{X} > 3$ ppb test another sample and accept the lot if $X \leqq 3$ ppb. Step 3: if $\bar{X} > 3$ ppb in the second sample test a third sample. If $\bar{X} \leqq 3$ ppb, accept the lot; if $\bar{X} > 3$ ppb reject the lot. The OC curve for this plan is shown in Figure 2.

Other attribute multiple-sampling plans may be developed which require a change in $\bar{X}$ and N for different trials. Using Table II the probabilities for each trial of the plan can be determined for the selected N and $\bar{X}$ values. For a plan consisting of only one success in T trials, the probability of accepting the lot with mean M may be computed as follow:

$$P(M) = p_1 + (1-p_1)\,p_2 + (1-p_1)\,(1-p_2)\,p_3 + \ldots + (1-p_1)\ldots(1-p_{T-1})\,p_T \qquad [7]$$

where the subscripts of the probabilities p are associated with the trial number.

Plan 4

The following sampling plan was used by the peanut industry for the 1969 crop. It consists of three steps: Step 1: test a 12,000 kernel sample from the lot; if $\bar{X} \leqq 4$ ppb, accept the lot. Step 2: if $\bar{X} > 4$ ppb in Step 1 test two additional 12,000 kernel samples; if $\bar{X} \leqq 2$ ppb for both samples accept the lot. Step 3: if $\bar{X} > 2$ ppb for either sample in Step 2, determine the average of the three sample means; if the average is less than or equal to 30 ppb accept the lot, otherwise reject the lot.

TABLE III

Probabilities of Accepting a Lot of Shelled Peanuts With Average Levels of Aflatoxin M With a Success Level of $\bar{X}_s$, S Successes From T Trials, and N Kernels per Sample[a]

		Sample size N					
		9000		12,000		15,000	
$\bar{X}_s$	S	T	P	T	P	T	P
.1 M	1	1	0.17	1	0.11	1	0.07
	2	2	0.03	3	0.03	5	0.04
.2 M	1	1	0.26	1	0.20	1	0.15
	2	2	0.07	2	0.04	2	0.02

[a] Probabilities were obtained from Tables of the Cumulative Binomial Distribution. The probabilities associated with S = T = 1 came from the Negative Binomial Distribution.

This plan is a modified attribute-sampling plan since the average of the three sample means is used as a test criterion in Step 3. Because of Step 3 the procedure to determine the overall probability of passing a lot by the three steps is complicated and will not be described here (Miller, personal communication). However, the OC curve for Plan 4 is given in Figure 2.

Analysis of Sampling Plans

Comparison of OC curves for Plan 1 and 2 in Figure 2 demonstrates a marked reduction in consumer risk (assuming $M_c = 30$ ppb, for illustrative purposes only) with a slight increase in processor risk when sample size is increased from N = 12,000 to N = 35,000. This suggests that a further increase in sample size greater than 35,000 kernels would be beneficial. Plan 3 represents an attempt to obtain more information per pound of sample. The OC curve for Plan 3 shows that it provides more protection for the consumer and less protection for the processor than Plan 2. As discussed below, many lots would be accepted on the basis of Step 1 or Step 2 in Plan 3 and fewer peanuts would be required for the testing program than for Plan 2.

In Plan 3, all lots would be tested by Step 1. The number of lots tested by Step 2 would be the total number of lots minus those accepted in Step 1. The number of lots tested by Step 3 would be the total number of lots less those accepted in Steps 1 and 2. The total number of tests by Plan 3 for all M values were computed with the following equation from the data presented in Table I.

$$T = \sum_{M=0}^{M=\infty} L(M)\ (3-3p+p^2) \qquad [8]$$

where L is the number of lots with a given M value and p is the probability of obtaining $\bar{X} \leqq 3$ ppb from the lots when N = 18,000. The average number of tests per lot is T/L and the average sample size is T/L × 18,000. These computations show that an average sample size of 31,242 kernels/lot is required for Plan 3 compared to 35,000 kernels/lot for Plan 2. The average number of samples per lot versus the true lot mean M for Plan 3 is plotted in Figure 3. The curve demonstrates a rapid increase in the number of samples per lot with an increase in M and suggests that the number of samples required for testing is greatly influenced by the prior distribution.

Equations 1 to 6, in conjunction with an estimated prior distribution and OC curves, can be used to estimate the number of lots associated with the consumers' and processors' risk and the average level of aflatoxin in the lots accepted. Table IV compares

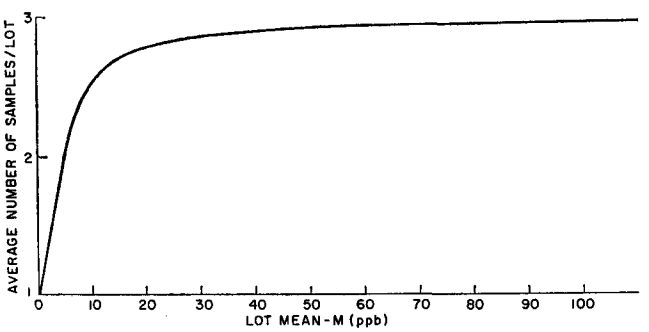

FIG. 3. Average number of samples per lot required by Sampling Plan 3, when the lots contain the indicated mean level of aflatoxin.

TABLE IV

Comparison of Sampling Plans 1–4

Basis of comparison	Plan No.			
	1	2	3	4
Per cent of all lots tested that will be accepted	86.4	84.5	73.2	90.3
Avg. Aflatoxin concentration of accepted lots (ppb)	4.8	3.4	2.1	5.2
Per cent of all lots accepted that have M ≦ 30 ppb	96.3	98.6	99.0	96.4
Per cent of all lots accepted that have M > 30 ppb	3.7	1.4	1.0	3.6
Per cent of good lots (M ≦ 30 ppb) accepted	94.0	94.1	81.9	98.3
Per cent of bad lots (M > 30 ppb) accepted	27.7	10.4	6.4	28.3
Avg. sample size required for test (number of kernels)	12,000	35,000	31,242	.....
Avg. no. trials per lot	1	1	1.7	

sampling Plans 1 through 4 using the estimated prior distribution shown in Table I. The Table indicates that for the sampling plans based on a single sample, Plan 2 provides both the producer and consumer more protection than Plan 1 but a larger sample is required. This demonstrates the desirable effect of increasing sample size. Comparison of Plans 2 and 3 demonstrates some of the advantages and disadvantages of multiple sampling. Plan 3 is better than Plan 2 in each category except the per cent of good lots accepted. This indicates that the processors' risk is higher with Plan 3. However, the consumers' protection is higher in Plan 3 even though the average amount of sample used to make a decision is less. Inspecting Plan 4 shows that it has the lowest processors' risk, but the consumers' risk is somewhat higher than in Plans 2 and 3.

The sampling plans analyzed in Table IV are used mainly for illustrative purposes. Also the data shown in Table IV applies only to the overall crop and is based on the assumption that the estimated prior distribution for the 1967 crop would be valid for other crop years. Experience of the peanut industry indicates that there probably are major fluctuations in the distribution from year to year and from one geographic region to another. These fluctuations have significant effects upon the performance of various sampling plans.

Critical evaluation and selection of a sampling plan for peanuts involve, among others, the following factors: (a) tolerable risks for the consumer and the processor; (b) the value of peanuts destroyed by testing and related costs of taking the sample, shipping, comminution and subsampling; (c) errors introduced by taking small subsamples from large primary samples and (d) cost of aflatoxin analyses. In addition, the OC curves, presented in this paper consider sampling error only. Further work is needed to compute OC curves based upon the combined error terms for sampling, subsampling, and the analysis of subsamples. Selection of an optimum sampling plan for the peanut industry depends upon quantification of these factors and their combined analysis.

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Lawrence Atkin, Scientific Director of Standard Brands, Inc. contributed to this study and expressed interest in its application to the peanut industry.

REFERENCES

1. Bampton, S. S., Trop. Sci. *5*, 74–81 (1963).
2. Whitaker, T. B., and E. H. Wiser, JAOCS *46*, 377–379 (1969).
3. Cuculu, A. F., L. S. Lee, R. Y. Mayne and L. A. Goldblatt, JAOCS *43*, 89–92 (1966).
4. Staff of the Computation Laboratory, Harvard University, "Tables of the Cumulative Binomial Probability Distribution," Harvard University Press, Cambridge, Massachusetts, 1955.

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