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Characteristics of Visual, Minicolumn and TLC Methods in Detecting Aflatoxin Contaminated Loads of Farmers Stock Peanuts

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ABSTRACT

Results from two 1980 experiments were used to compare the performance of the visual, minicolumn and thin-layer chromatography (TLC) methods in detecting loads of farmers stock peanuts with aflatoxin. The first experiment was conducted to establish variability of the methods under ideal conditions. The second experiment was conducted to evaluate the performance of the three methods under commercial conditions. In this paper, data and empirical models were used to explore potential improvements in detecting low-level contaminated lots. The minicolumn and TLC methods were very sensitive and provided consistent measurements. Operation characteristic (OC) curves developed by using empirical models (Logistic and Gompertz) were compared to statistical distribution functions used by Whitaker and coworkers. Assuming a desired probability of acceptance of 15%, both the minicolumn and TLC methods would accept lots with 60 ppb aflatoxin, while the visual methods would accept lots with 150 ppb aflatoxin. In crop years similar to 1980, the currently used method would not segregate peanuts to meet the 1-5 ppb (total) tolerance levels and would provide excessive sheller risk. Because of their objectivity, precision and higher sensitivity, the minicolumn and TLC have potential for improving the detection of contaminated loads of farmers stock peanuts.

Key Words: Farmers stock peanuts, aflatoxin, sampling, operation characteristics (OC) curves.

Peanut farmers in the U.S. usually produce more aflatoxin-free peanuts than are needed for the domestic edible and export trade. Exceptions were in the drought years of 1972, 1980 and 1984. A provision of the U. S. Department of Agriculture Peanut Marketing Agreement (9) requires that certain kernels from each sample of farmers stock peanuts (*Arachis hypogaea* L.) be examined for visible growth of the aflatoxin-producing fungi, *Aspergillus flavus* and *A. parasiticus* (4). Lots with samples found to contain one or more kernels with visible *A. flavus* or *A. parasiticus* mold growth are classified as Segregation (Seg.) III. These peanuts are processed for oil, and the meal is restricted to non-feed uses.

Use of the current sample size and visual *A. flavus* examination method (VAF) can result in storage of contaminated lots with aflatoxin-free lots (2). Once the mixing in storage occurs, contaminated kernels cannot be removed except by a costly blanching and extensive sorting process (6-15% material loss and $80/ton blanching cost). Even with a 25 ppb tolerance level, the industry spends several million dollars each year in indemnification, blanching and remilling (11).

Since aflatoxin acceptance levels in many foreign countries are 1-5 ppb (total), U.S. peanuts often fail to meet these acceptance levels. Consequently, there is an urgency in studying, evaluating and recommending needed changes and/or improvements for the current aflatoxin control program for farmers stock and shelled peanuts.

Mathematical studies of the distribution of aflatoxin in shelled peanuts have been conducted. Negative binomial distribution was first used by Whitaker *et al.* (16,17) in estimating the probabilities associated with sampling lots of shelled peanuts for aflatoxin analysis. On the basis of small samples and the TLC method, Knutti and Schlatter (8) compared a compound Poisson-Gamma distribution to a negative binomial distribution and considered the negative binomial distribution a representative statistical model for levels of 0-200 ppb. Brown (1), using the TLC method, found the negative binomial model did not adequately fit the observed frequency of samples with 0-100 ppb aflatoxin, and an alternative lognormal distribution was suggested.

In a cooperative study in 1980, USDA researchers conducted two experiments to compare VAF, minicolumn (MCL) and modified thin-layer chromatography (TLC) for detecting small lots of farmers stock peanuts with varying aflatoxin concentrations. Results (2) showed that the overall performance of the three methods were similar except at the maximum sensitivity level (< 1 ppb and <1 *A. flavus* kernel). At maximum sensitivity, the TLC and MCL methods were more effective than the current VAF method in detecting lots with aflatoxin. Subsequently, Whitaker *et al.* (14,15) developed statistical models using the negative binomial

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probability function and Poisson probability function to further compare the performance of the MCL, TLC and VAF methods at various critical levels above the maximum sensitivity levels ($\geq$ 1 ppb and $\geq$ 1 *A. flavus* kernel).

In this paper, efforts are made to systematically examine the available data and evaluate the feasibility of the VAF, MCL and TLC methods to detect aflatoxin in farmers stock peanuts. The purpose of this and future papers is to explore potential improvements in detecting strategies where lower aflatoxin levels are desired. Specific objectives include (1) using data from the first experiment to develop OC curves based on different mathematical equations for the VAF, MCL and TLC methods when using the current grade sample size for aflatoxin analysis; (2) comparing the efficiency of the three methods in terms of OC curve performance at maximum sensitivity; and (3) using data from 2297 commercial loads in the second experiment to analyze the agreement and/or disagreement among the three methods in actual commercial applications.

Materials and Methods

Experiment I

Experiment Design. Details of this experimental study have been published by Davidson *et al.* (2). A brief discussion of these procedures follows. During the crop year 1980, 40 separate lots of farmers stock peanuts were selected from lots identified as Set. III by the VAF method (5). To minimize sampling errors, a large minilot (approximately 61 kg) was taken from each of the lots with a Federal-State Inspection Service pneumatic sampler. As diagrammed by Davidson *et al.* (2), each minilot was divided into two equal portions (half A and half B) by using the Federal-State Inspection Service farmers stock divider. The foreign material was removed from half A of each lot. Loose shelled kernels (LSK) and inshell peanuts were separated to permit independent aflatoxin analysis using the standard TLC method (13). The test results of half A were used to select 20 of the 40 minilots that had estimated aflatoxin levels within the 5 to 300 ppb range. Half B of the minilot from each of the 20 selected lots was divided into 16 official grade samples weighing approximately 465 g. The number of kernels or kernel pieces with visible *A. flavus* growth in each grade sample were determined, and the aflatoxin concentrations were determined by TLC and MCL methods (7). The remaining portion of half B not used for grade samples was also evaluated for aflatoxin by the standard TLC method. The weighted mean aflatoxin concentration of each minilot was thus calculated from the TLC assay values of half A, the remaining of half B and the 16 grade samples.

Mathematical Analysis. The performance of a testing program can be best portrayed by the OC curve, which is a relationship between the probability of accepting the lot and the lot concentration. In general, an OC curve is an S-shaped curve that approaches 1 as aflatoxin concentration (ppb) becomes zero, and approaches zero as aflatoxin concentration becomes large. In order to develop OC curves to simulate the aflatoxin detecting methods, these characteristics of the curve were considered.

The percent of samples reported by Davidson *et al.* (2) indicating rejection (100 - acceptance) from each of the 20 minilots when either of 4 different acceptance levels was used for VAF, MCL and TLC methods are presented in Table 1. This set of experimental data was used to develop OC curves that describe the feasibility in detecting aflatoxin in lots of farmers stock peanuts by each of the three methods.

Based on the general curve shape, the logistic function (Equation 1) and Compertz function (Equation 2) (6) were selected to denote the OC curves for the three methods. With the logistic function, the probability (Prob) of accepting a lot with an aflatoxin concentration X (ppb) is calculated as

$$\text{Prob}(X) = 1 - \frac{\tau}{1 + \alpha(\beta)\sqrt{X}} \quad (1)$$

Table 1. Percent of samples from each of the 20 minilots indicating rejection when using various critical acceptance levels.[a]

Minilot No.	Aflatoxin concentration of minilot ppb	VAF method acceptance levels (No. kernels)				TLC method acceptance levels (ppb)				MCL method acceptance levels (ppb)			
		0	1	2	3	0	25	50	100	0	25	50	100
1	8	12	12	0	0	44	12	6	6	31	19	19	12
2	10	25	25	0	0	31	19	6	6	38	19	12	6
3	14	50	31	25	19	80	33	20	13	81	44	12	6
4	21	75	75	56	31	56	25	12	6	56	38	31	12
5	25	12	12	6	0	80	40	20	13	50	31	19	6
6	27	6	6	6	0	38	12	6	6	44	12	6	6
7	34	62	62	12	0	81	50	44	31	81	62	56	25
8	42	69	62	31	12	69	50	31	25	75	56	44	44
9	42	38	19	12	-	56	19	19	19	19	12	12	12
10	59	81	62	31	12	88	75	56	25	94	81	81	50
11	64	69	62	25	12	94	75	62	31	88	69	62	25
12	111	75	62	44	25	94	80	73	33	94	81	75	44
13	128	69	62	56	31	100	81	69	44	100	94	69	44
14	157	100	100	75	75	100	94	75	50	100	88	88	69
15	166	100	100	100	94	100	94	81	81	100	100	94	75
16	166	81	81	62	56	94	81	62	62	100	94	88	69
17	179	100	100	100	100	100	94	94	81	100	94	94	88
18	198	100	94	94	88	100	87	67	60	100	100	88	69
19	242	88	88	75	38	88	81	69	50	94	88	88	81
20	255	100	100	100	81	100	100	87	67	100	100	94	81

[a]The acceptance level is either the maximum number of peanut kernels (or pieces with visible A. flavus growth or the maximum concentration of aflatoxin in the sample before rejection of the lot.

where α, β, and τ are regression parameters specific for each detecting method and acceptance level. With the Compertz function, the probability is

$$\text{Prob}(X) = 1 - \epsilon \exp\left[-\theta e^{-K\sqrt{X}}\right] \quad (2)$$

where ϵ, θ, and K are also regression parameters for a specific method and acceptance level. Both functions produce S-shaped curves typical of the OC curves.

Experimental data was fitted to Equations (1) and (2) and to the negative binominal and Poisson functions as described by Whitaker *et al.* (15,17). The NLIN regression procedure (12) for non-linear models was used to determine the parameters of the equations. A square root transformation of lot aflatoxin concentration was used to fit the logistic and Gompertz equations. The sums of squared deviations were calculated and compared to evaluate Eqs. 1 and 2 as well as the Poisson and negative binomial distributions (15) in describing the OC curves. The empirical models are much more limited than the statistical models in extrapolating their usage to other data sets. However, these models may be more descriptive for commercial applications.

Experiment II

The objective of Experiment II was to compare the VAF method with the MCL method when using the maximum sensitivity levels on a large number (2297) of loads. During crop year 1980 harvest season, 2087 grade samples of Seg. I and 210 grade samples of Seg. III peanuts, as identified by the VAF method, were collected at buying points throughout Georgia. Each of the 2297 samples was analyzed by the MCL method (7) to determine aflatoxin concentration. To minimize the number of analyses, TLC and high pressure liquid chromatography (HPLC) analyses were conducted only on selective

samples to confirm test results as follows. It was assumed that TLC and HPLC analyses would agree with the VAF and MCL methods when they were in agreement. Therefore, only samples of Seg. III peanuts with a negative MCL (aflatoxin-free) and those of Seg. I peanuts with a positive MCL (aflatoxin found) were then analyzed by TLC and HPLC methods.

Data obtained were analyzed to determine the distribution of the samples according to six possible agreement or disagreement categories relative to the presence and absence of contamination as indicated by the VAF, MCL and TLC methods. Within each of the categories, the distribution of aflatoxin concentration of samples according to MCL and TLC analyses were determined.

Results and Discussion

Experiment I

The average percentage of samples showing positive test results for VAF, TLC and MCL methods was calculated from Table 1 and presented in Table 2. The percent of positive test results for the TLC and MCL methods were greater than for the VAF method (78% and 77% vs 66%). On the average, the sensitivity of 0 VAF kernels was equivalent to that of 15 ppb for both TLC and MCL methods.

Table 2. Average percentage of contaminated samples as indicated by the VAF, TLC and MCL methods when operating at maximum sensitivity levels.

Methods	Mean (%)	Standard deviation (%)	Equivalent sensitivity of VAF (%)
VAF	66	32	66 (0 kernel)
TLC	78	23	67 (15 ppb)
MCL	77	27	69 (15 ppb)

The parameters for Eqs. 1 and 2 are shown in Table 3 with the sum of squared deviations for goodness of fit comparisons. Also shown are the sum of squared deviations for the negative binormal probability function (TLC and MCL methods) and Poisson probability function (VAF method) developed by Whitaker *et al.* (17). A square root transformation of lot aflatoxin concentration was necessary to best fit the data in the models shown in Eqs. 1 and 2. The NLIN procedure showed that some of the parameters in the models could be approximated by constants. β and τ were set at 0.7 and 12.0 for the logistic function, and K was set at 0.5 for the Gompertz function. Equations with fewer parameters are more useful and easier to verify. There was substantial variability in the remaining parameter values in the equations between different critical acceptance levels and various aflatoxin testing methods. Using these parameters, OC curves of the various aflatoxin testing methods were developed.

The least sum of squared deviations (Table 3) indicated that the degree of goodness of fit for the TLC and MCL data were better than that for the VAF data regardless of empirical and statistical functions used. This suggests that the TLC and MCL methods would provide more consistent and predictable results than for the VAF method. Such results were expected since the TLC and MCL methods are less subjective and more analytical than the VAF method.

Table 3. The parameters and comparison of operating characteristic curves derived from experimental sample data and statistical sampling distributions used by Whitaker et al.

Critical acceptance level	Parameters: Logistic α	Parameters: Gompertz ϵ	Parameters: Gompertz θ	Least sum of squares: Logistic	Least sum of squares: Gompertz	Least sum of squares: Statistical
VAF						
0	8.62	0.897	9.068	0.588	0.688	0.749
1	11.88	0.863	11.043	0.608	0.779	0.973
2	32.27	0.873	52.433	0.459	0.655	0.576
3	60.81	0.849	147.564	0.660	0.593	0.651
TLC						
0	3.43	0.951	4.110	0.289	0.332	0.403
25	11.84	0.893	13.453	0.254	0.294	0.266
50	22.84	0.779	18.944	0.295	0.198	0.348
100	60.88	0.613	26.112	0.279	0.244	0.273
MCL						
0	4.33	0.952	5.080	0.491	0.591	0.584
25	10.05	0.932	12.525	0.362	0.470	0.377
50	14.81	0.894	17.529	0.328	0.337	0.338
100	40.19	0.726	27.585	0.202	0.269	0.292

The performance in overall fit of logistic function and the Gompertz functions to test data for a specific method and sensitivity level was similar (Table 3). However, the goodness of fit depended on the contamination levels (Figs. 1,2,3). When contamination levels were less than 60 ppb, the OC curves developed from the Gompertz and statistical models were in agreement.

As shown in Fig. 4, the MCL and TLC methods were in good agreement and demonstrated considerable potential for reducing the number of contaminated lots accepted when using the maximum sensitivity levels. For example, selecting a probability of acceptance of

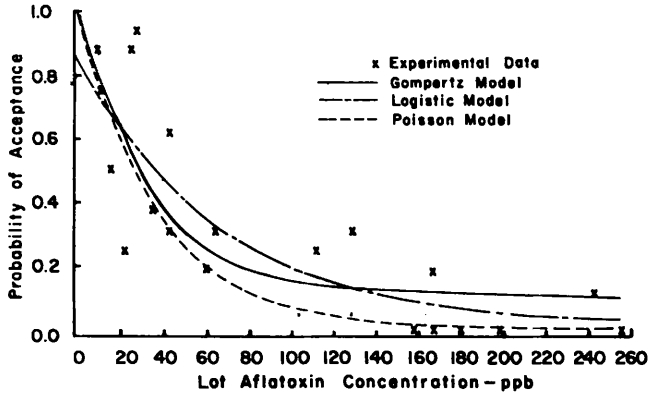

Fig. 1. Observed and predicted probability of acceptance associated with the VAF method at a critical acceptance level of 0 VAF kernels.

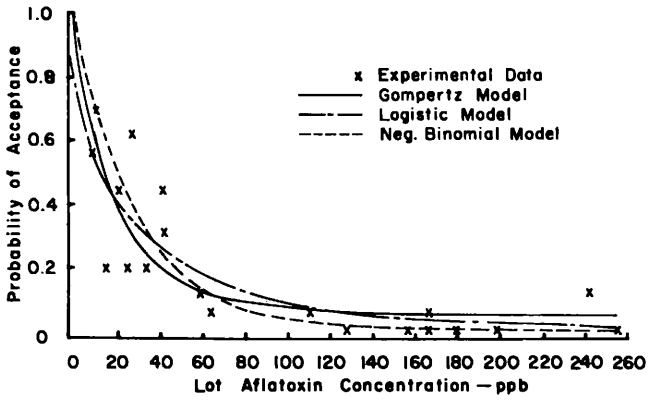

Fig. 2. Observed and predicted probability of acceptance associated with the TLC method at a 0 ppb critical acceptance level.

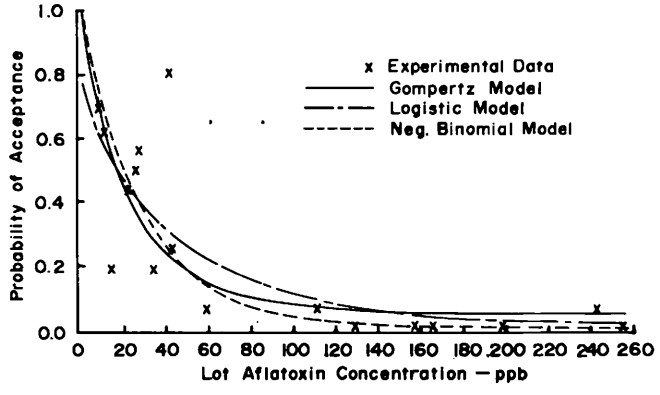

Fig. 3. Observed and predicted probability of acceptance associated with the MCL method at a 0 ppb critical acceptance level.

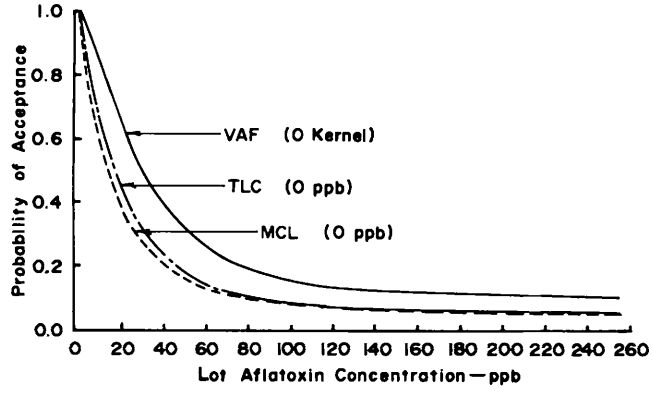

Fig. 4. Operating characteristic curves fitted to the Gompertz model for VAF, TLC and MCL methods at 0 critical acceptance level.

15%, both the MCL and TLC methods would accept contaminated lots with aflatoxin concentration less than 60 ppb, while the VAF method would accept lots with aflatoxin levels as high as 150 ppb.

Experiment II

Table 4 summarizes the results of this study. Based on a zero acceptance level, the MCL method identified more samples having aflatoxin than the VAF method. Using the TLC method as a check, the MCL agreed

$$\left(\frac{2233}{2297}\right) * 100 = 97.2\% \text{ of the samples.}$$

while the VAF method agreed

$$\left(\frac{1866}{2297}\right) * 100 = 81.2\% \text{ of the samples.}$$

Assuming the minicolumn is a reliable method, agreement with TLC was expected since a portion of the same extract was used by both methods.

Table 4. A comparison of the VAF and the MCL methods (using the TLC method as a check) at maximum sensitivity levels to detect *aflatoxin contaminated lots of farmers stock peanuts in 1980.*

Category	Methods VAF+	MCL	TLC (check)	No. of Samples	% of Sample+ Seg. I	Seg. III	Total	Mean aflatoxin concentration MCL	TLC
								ppb	
I	0	0	†	1613	77.3	-	70.2	0	-
II	0	>0	0	58	2.8	-	2.5	35	0
III	0	0	>0	416	19.9	-	18.1	81	136
IV	0	0	0	15	-	7.3	0.7	0	0
V	>0	0	>0	6	-	2.9	0.3	0	81
VI	>0	>0	†	189	-	90.0	8.2	833	-

+Samples of 0 visible A. flavus kernels (VAF) are Segregation I.
Samples of >0 visible A. flavus kernels (VAF) are Segregation III.

†Assumption was made that TLC would agree with both VAF and MCL methods.

As to agreement/disagreement between the two methods, approximately 1 out of 10 Seg. III samples were identified as Seg. I by the MCL method. The VAF and MCL methods were in agreement 90% of the time on Seg. III peanuts, but only 77.3% of the time on Seg. I peanuts. On the disagreement of Seg. I samples, only 2.8% of the Seg. I samples were identified by the MCL method as being contaminated and then found to be negative by TLC method. The MCL and TLC methods concurred in identifying 19.9% of the Seg. I samples that were contaminated. This higher probability of acceptance of contaminated lots with the VAF method agrees with industry data (10).

As estimated by the MCL method, the weighted mean aflatoxin for all samples was 84 ppb. The weighted mean aflatoxin of Seg. III and Seg. I samples were 750 ppb and 17 ppb, respectively. Thus, the critical acceptance level of 0 VAF kernels was not sensitive enough to detect existence of aflatoxin at low contamination levels. This finding explains the difficulty experienced by the peanut industry in meeting the export market tolerance levels of 1-5 ppb. If the MCL method was used to determine segregation of peanuts, the estimation of aflatoxin level of Seg. III samples would be 291 ppb.

Table 4 showed 19.9% of Seg. I samples (Category III) had aflatoxin levels of 81 and 136 ppb as determined by the MCL and TLC methods, respectively. Of these samples, approximately 60% had aflatoxin levels higher than 25 ppb, and over 30% had aflatoxin levels higher than 100 ppb. Those Seg. I samples that tested positive by the MCL method but negative by the TLC method (Category II) had average concentrations of 35 and 21 ppb as determined by the MCL and HPLC methods, respectively. In addition, 75% of these samples had aflatoxin concentration <25 ppb by the HPLC method.

Thus, there would be very few samples identified as Seg. III by the MCL that would not have aflatoxin. Also, an analysis of Category V samples indicated that the TLC average value for five of the six samples was 22 ppb, and HPLC determinations for all six samples average 17 ppb. As a result, in considering the use of the MCL for testing Seg. III peanuts, approximately 10% of the Seg. III samples would be found to be Seg. I with an accuracy of 71% (i.e. 71% of the Seg. III samples identified as Seg. I by the MCL would not have aflatoxin). All of the other 30% essentially had levels of aflatoxin below 25 ppb as determined by the TLC and HPLC.

Two kinds of risks are associated with an aflatoxin testing program for farmers stock peanuts. Using the TLC assay as a standard, the risk of a good lot testing bad for the MCL method (farmer's risk) was 2.5%. The risk of a bad lot testing good (sheller's risk) was 0.3%. Conversely, for the VAF method the farmer's risk was 0.7% and the sheller's risk was 18.1%. Future testing programs should involve compromising and reducing both risk by using larger sample sizes and using improved sampling, segregating suspect kernels (3) and use of more sensitive detection methods such as MCL and TLC methods.

Summary and Conclusions

The results show that the MCL and TLC methods were in good agreement as to the absence or presence of aflatoxin. These two methods were more sensitive and predictable than the VAF method for detecting lots contaminated with aflatoxin. Using the current sample size, a sensitivity level of 0 VAF kernels is approximately equivalent to 15 ppb by the two analytical methods. The degree of goodness of fit showed that the logistic and Gompertz as well as statistical models tended to provide a better fit to results of MCL and TLC methods than that of the VAF method. Also, Gompertz and logistic models tended to provide higher acceptance probabilities of contaminated lots than for the statistical models. Improved models that fit commercial data are needed to assess risks and provide information for reducing aflatoxin in peanuts. The MCL and TLC methods have good potential for improving the detection of contaminated loads of farmers stock peanuts. Such methods together with an alternative aflatoxin control and testing program could provide better quality peanuts, increase peanut exports and provide a safer and cheaper product to the consumer.

Literature Cited

1. Brown, G. H. 1984. The distribution of total aflatoxin levels in composited samples of peanuts. Food Techn. Aust. 36(3):128-130.
2. Davidson, J. I., Jr., J. W. Dickens, V. Chew, T. H. Sanders, C. E. Holaday, R. J. Cole and T. B. Whitaker. 1985. Performance of the visual, minicolumn and TLC methods in detecting aflatoxin in 20 contaminated lots of farmers stock peanuts. Peanut Sci. 11:77-83.
3. Davidson, J. I., Jr., C. E. Holaday and C. T. Bennett. 1981. Separation and removal of aflatoxin contaminated kernels in peanut shelling plants: Part I. A case study. Proc. Am. Peanut Res. Educ. Soc. 13:29-45.
4. Dickens, J. W. 1977. Aflatoxin control program for peanut. J. Am. Oil Chem. Soc. 54:225A-227A.
5. Dickens, J. W. and J. B. Satterwhite. 1971. Diversion program for farmers stock peanuts with high concentrations of aflatoxin. Oleagineux 26:321-328.
6. Draper, N. R. and H. Smith. 1981. Chapter 10. Applied Regression Analysis. John Wiley and Son, New York, pp. 511.
7. Holaday, C. E. 1976. A rapid screening method for the aflatoxins and ochratoxin A. J. Am. Oil Chem. Soc. 53:603-605.
8. Knutti, R. and C. Schlatter. 1982. Distribution of aflatoxin in whole peanut kernels, sampling plans for small samples. Z. Lebensm. Unters. Forsch. 174:122-128.
9. Marketing Agreement for Peanuts, No. 146. 1984. Peanut Administrative Committee, P. O. Box 18856, Atlanta, GA 30326.
10. National Peanut Council Peanut Quality Task Force Report. December 1987. National Peanut Council, 101 S. Peyton St., Suite 301, Alexandria, VA 22314.
11. Peanut Administrative Committee Annual Report. 1987. Peanut Administrative Committee, P. O. Box 18856, Atlanta, GA 30326.
12. SAS User's Guide. 1985. SAS Institute, INC., Box 800, Cary, NC 27511.
13. U. S. Department of Agriculture Inspectors instruction for aflatoxin. 1975. USDA, Agricultural Marketing Service, Washington, D.C.
14. Whitaker, T. B. and J. W. Dickens. 1986. Efficacy of the visual, minicolumn and TLC methods to test farmers stock peanuts for aflatoxin. Peanut Sci. 13:74-77.
15. Whitaker, T. B., J. W. Dickens and V. Chew. 1985. Development of statistical models to simulate the testing of farmers stock peanuts for aflatoxin using visual, TLC and minicolumn methods. Peanut Sci. 12:94-98.
16. Whitaker, T. B., J. W. Dickens and R. J. Monroe. 1974. Variability of aflatoxin test results. J. Am. Oil Chem. Soc. 51:214-218.
17. Whitaker, T. B., J. W. Dickens, R. J. Monroe and E. H. Wiser. 1972. Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J. Am. Oil Chem. Soc. 49:590-593.

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