Variability Associated with Testing Corn for Aflatoxin[1]

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ABSTRACT

The sampling, subsampling (both coarse and fine ground meal), and analytical variances associated with testing shelled corn for aflatoxin were estimated by the use of 500 g samples, 50 g subsamples, and the CB method of analysis. The magnitudes of the variance components increased with an increase in the aflatoxin concentration. Functional relationships were developed to predict the variance for a given aflatoxin concentration and any size sample, subsample, and number of analyses. At 20 ppb total aflatoxin, the coefficient of variantion associated with a 4.54 kg sample, 1 kg subsample of coarsely ground meal (passes a #14 screen), a 50 g subsample of finely ground meal (passes a #20 screen) and one analysis were 21, 8, 11, and 26%, respectively.

INTRODUCTION

The aflatoxin concentration in a lot of shelled corn may be estimated from the concentration of aflatoxin in a sample drawn from the lot. The AOAC Official First Action Method for corn (1) does not specify sample size, but it requires that the entire sample be ground to pass a No. 14 sieve (coarse grind), and that a 1–2 kg subsample of this material be ground to pass a No. 20 sieve (fine grind). A 50 g subsample of the fine grind is then analyzed. The total error associated with this test may consist of errors related to each of the following steps: (a) sampling the lots of shelled corn (sampling error), (b) subsampling the coarse-grind material (coarse subsampling error), (c) subsampling the fine-grind material (fine subsampling error), and (d) determining the aflatoxin concentration in the 50 g subsample (analytical error). These errors are diagrammed in Figure 1. An aflatoxin test result $\bar{x}$ may be represented as follows:

$$\bar{x} = \mu + s + c + f + a \qquad 1.$$

where μ is the true aflatoxin concentration in the lot of shelled corn being tested; s is the random error due to sampling with a mean value of zero and variance $\sigma^2_{\bar{x}(s)}$; c is the random error due to coarse subsampling with a mean value of zero and variance $\sigma^2_{\bar{x}(c)}$; f is the random error due to fine subsampling with a mean value of zero and variance $\sigma^2_{\bar{x}(f)}$; and a is the random error due to analysis with a mean value of zero and variance $\sigma^2_{\bar{x}(a)}$. If both stochastic and functional independence among the random errors in equation 1 are assumed, the following variance relationship is obtained:

$$\sigma^2_{\bar{x}(t)} = \sigma^2_{\bar{x}(s)} + \sigma^2_{\bar{x}(c)} + \sigma^2_{\bar{x}(f)} + \sigma^2_{\bar{x}(a)} \qquad 2.$$

where $\sigma^2_{\bar{x}(t)}$ is the total variance associated with the aflatoxin test result $\bar{x}$. Since previous studies on peanuts have indicated that the variance components in equation 2 are functionally related to the aflatoxin concentration, the assumption concerning the nature of the random errors s, c, f, and a may be open to question. However, other statistical models, such as the multiplicative model, were investigated but did not provide a workable alternative.

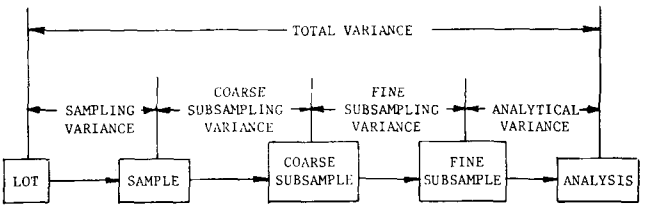

FIG. 1. Typical steps used to estimate the aflatoxin concentration μ in shelled corn and the associated sources of variation.

The objective of this study was to quantify empirically the sampling, coarse subsampling, fine subsampling, and analytical variances associated with testing shelled corn for aflatoxin.

EXPERIMENTAL PROCEDURE

Collection of Material

Samples of shelled corn weighing ca. 1 kg each were collected from ca. 400 different commercial lots of corn by the grain division of the North Carolina Department of Agriculture during 1976 and 1977. The corn was combined and subdivided to form 10 small lots, called minilots, each weighing ca. 40 kg. It was assumed that the distribution of alfatoxin-contaminated corn kernels in the minilots was typical of the distribution which would be found in the average commercial lot.

Sampling and Subsampling

Sixty-four 500 g samples were prepared from each minilot, and the samples contained an average of 3,050 kernels per kilogram. Each sample of shelled corn was comminuted by a subsampling mill with a 3.2 mm screen similar to the mills used for peanuts in most aflatoxin laboratories (2). Over 99 percent of the comminuted material passed through a number 14 sieve. A sieve analysis indicated that the average particle diameter was 430 microns (3,4). A further particle size reduction was obtained by grinding the comminuted meal in a Wiley mill with a 1 mm screen. The Wiley mill reduced the particle size so that over 99 percent of the particles passed through a number 20 sieve. A sieve analysis indicated that the average particle diameter was 240 microns.

Chemical Analysis

Aflatoxin was extracted from 50 g subsamples of the finely ground corn by the AOAC Official First Action Method (CB Method) (1) and fluorescent intensities on TLC plates were quantified densitometrically. All analyses were made in the same laboratory.

Data Analysis

Only the analytical variance component $\sigma^2_{\bar{x}(a)}$ in equa-

[1]Paper number 5661 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27650.

tion 2 can be estimated independently. It is not possible to estimate $\sigma^2_{\bar{x}(s)}$, $\sigma^2_{\bar{x}(c)}$, and $\sigma^2_{\bar{x}(f)}$ independently because the variance due to analysis is involved in every test result. Therefore, the combined sampling plus analytical variance $\sigma^2_{\bar{x}(sa)}$, combined coarse subsampling pluse analytical variance $\sigma^2_{\bar{x}(ca)}$, and combined fine subsampling plus analytical variance $\sigma^2_{\bar{x}(fa)}$ were estimated where

$$\sigma^2_{\bar{x}(sa)} = \sigma^2_{\bar{x}(s)} + \sigma^2_{\bar{x}(a)} \quad 3.$$

$$\sigma^2_{\bar{x}(ca)} = \sigma^2_{\bar{x}(c)} + \sigma^2_{\bar{x}(a)} \quad 4.$$

and

$$\sigma^2_{\bar{x}(fa)} = \sigma^2_{\bar{x}(f)} + \sigma^2_{\bar{x}(a)} \quad 5.$$

Once values of $\sigma^2_{\bar{x}(sa)}$, $\sigma^2_{\bar{x}(ca)}$, $\sigma^2_{\bar{x}(fa)}$, and $\sigma^2_{\bar{x}(a)}$ are available, then $\sigma^2_{\bar{x}(s)}$, $\sigma^2_{\bar{x}(c)}$, and $\sigma^2_{\bar{x}(f)}$ can be computed from equations 3, 4, and 5. The total variance $\sigma^2_{\bar{x}(t)}$ can be computed from equation 2. Experimental estimates of $\sigma^2_{\bar{x}}$ and μ are denoted by $S^2_{\bar{x}}$ and $\bar{\bar{x}}$ where $\bar{\bar{x}}$ is the average of observed $\bar{x}$ values.

Theoretical Considerations

It was assumed that the variance components $\sigma^2_{\bar{x}(a)}$, $\sigma^2_{\bar{x}(s)}$, $\sigma^2_{\bar{x}(c)}$, and $\sigma^2_{\bar{x}(f)}$ are zero when aflatoxin concentration μ is zero. Previous studies (5,6) indicate that the analytical variance $\sigma^2_{\bar{x}(a)}$ can be described by the following equation

$$\sigma^2_{\bar{x}(a)} = c_1 \mu^2 \quad 6.$$

The negative binomial function (7,8) has been used to describe the distribution of aflatoxin-contaminated kernels in shelled peanuts and cottonseed (9,10); so it was assumed that this distribution is applicable to corn. With the negative binomial function, the variance among the individual members of the population σ^2 is related to the mean of the population μ as follows:

$$\sigma^2 = \mu + (\mu^2/k) \quad 7.$$

where k is the shape parameter. If replicate samples of ns items are drawn from the lot, then the variance $\sigma^2_{\bar{x}(s)}$ among sample concentrations $\bar{x}$ is related to σ^2 as follows:

$$\sigma^2_{\bar{x}(s)} = \sigma^2/ns \quad 8.$$

Substituting equation 8 into 7 gives

$$\sigma^2_{\bar{x}(s)} = (1/ns)(\mu + (\mu^2/k)) \quad 9.$$

It has been demonstrated with peanuts (5) that k could be linearly related to the mean concentration μ.

$$k = c_2\mu \quad 10.$$

Substituting equation 10 into 9 and simplifying gives

$$\sigma^2_{\bar{x}(s)} = B_1\mu \quad 11.$$

where

$$B_1 = (1/ns)(1 + (1/c_2)) \quad 12.$$

and ns is the number of kernels in samples drawn from the lot. It can be seen from equation 3 that the combined sampling and analytical variance can be related to the aflatoxin concentration by adding together the analytical and sampling variances in equations 6 and 11. This gives

$$\sigma^2_{\bar{x}(sa)} = B_1\mu + c_1\mu^2 \quad 13.$$

In accordance with previously mentioned work on peanuts and cottonseed, the negative binomial function was also used to describe the distribution among contaminated particles in the coarse and fine ground samples. The equations take a form similar to that given above for sampling. The equation for the combined coarse subsampling and analytical variance is

$$\sigma^2_{\bar{x}(ca)} = B_2\mu + c_1\mu^2 \quad 14.$$

where

$$B_2 = (1/nc)(1 + (1/c_3)) \quad 15.$$

nc is the weight of the coarsely ground subsamples, and c_3 is the proportionality constant between the shape parameter k_c and the aflatoxin concentration μ.

The equation for the combined fine subsampling and analytical variances takes a form similar to equations 13 and 14.

$$\sigma^2_{\bar{x}(fa)} = B_3\mu + c_1\mu^2 \quad (16).$$

where

$$B_3 = (1/nf)(1 + (1/c_4)) \quad 17.$$

nf is the weight of the finely ground subsamples, and c_4 is the proportionality constant between the shape parameter k_f and μ.

The coefficients c_1, B_1, B_2, and B_3 in equations 6, 13, 14, and 16 can be determined by regression techniques. With known values of B_1, B_2, B_3, ns, nc, and nf, the proportionality constants c_2, c_3, and c_4 can be computed from equations 12, 15, and 17.

Analytical Variance

The analytical variance $S^2_{\bar{x}(a)}$ is defined as the variance among aflatoxin determinations on equal quantities of extract taken from the filtration step spedified in the AOAC Official First Action Method 1 (CB Method) (1). A 1600 g sample of finely ground material was prepared from each of the 10 minilots and divided into thirty-two 50 g subsamples. Aflatoxin in each of the 32 subsamples was extracted as specified by the CB Method. Extracts, ca. 65 ml,

from each subsample was pooled (a total of 2080 ml) and blended. The aflatoxin concentration in each of thirty-two 50 ml portions of the blended extract was determined by the remaining steps of the CB Method. The variance $S^2_{\bar{x}(a)}$ and the average $\bar{\bar{x}}$ of the 32 test results were calculated for each of the 10 minilots.

Combined Sampling and Analytical Variance

The combined sampling and analytical variance $S^2_{\bar{x}(sa)}$ is defined as the variance among aflatoxin determinations on samples from the same minilot of shelled corn when the aflatoxin is extracted from the entire contents of each sample by the CB Method. With a riffle divider, thirty-two 500 g samples were subdivided from each of 7 minilots. Each 500 g sample of shelled corn was ground in a Wiley mill with a 1 mm screen. The meal from each sample was divided into ten 50 g subsamples, and each subsample was taken through the extraction step of the CB Method. A composite blend was made from a 50 ml portion of extract from each of the 10 subsamples. One determination of the aflatoxin concentration in a 50 ml portion from each of the 32 composite blends of extract was then made according to remaining steps of the CB procedure. The combined sampling and analytical variance $S^2_{\bar{x}(sa)}$ and the average $\bar{\bar{x}}$ of the 32 test results (one for each sample) were calculated for each of the 7 minilots.

Combined Coarse Subsampling and Analytical Variance

The combined coarse subsampling and analytical variance $\sigma^2_{\bar{x}(ca)}$ is defined as the variance among aflatoxin determinations on subsamples taken from a sample of corn coarsely ground in the subsampling mill with a 3.2 mm screen. A 1600 g sample of coarse ground material from each of 7 minilots was riffle-divided into thirty-two 50 g subsamples. Each subsample was comminuted in the Wiley mill, and the aflatoxin concentration was determined by the CB Method. The variance $S^2_{\bar{x}(ca)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ of the 32 test results were calculated for each of the 7 minilots.

Combined Fine Subsampling and Analytical Variance

The combined fine subsampling and analytical variance $\sigma^2_{\bar{x}(fa)}$ is defined as the variance among aflatoxin determinations on subsamples taken from a sample of corn comminuted in a Wiley mill with a 1 mm screen. A 1600 g subsample of the coarse ground material from each of 7 minilots was ground in the Wiley mill. The 1600 g of finely ground material was riffle-divided into thirty-two 50 g subsamples. The aflatoxin concentration in each subsample was determined by the CB Method. The variance $S^2_{\bar{x}(fa)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ of the 32 test results were calculated for each of the 7 minilots.

Sequence of Analysis

The analytical variance had to be subtracted from the combined variances shown in equations 3, 4, and 5 if the sampling and subsampling variances were to be determined.

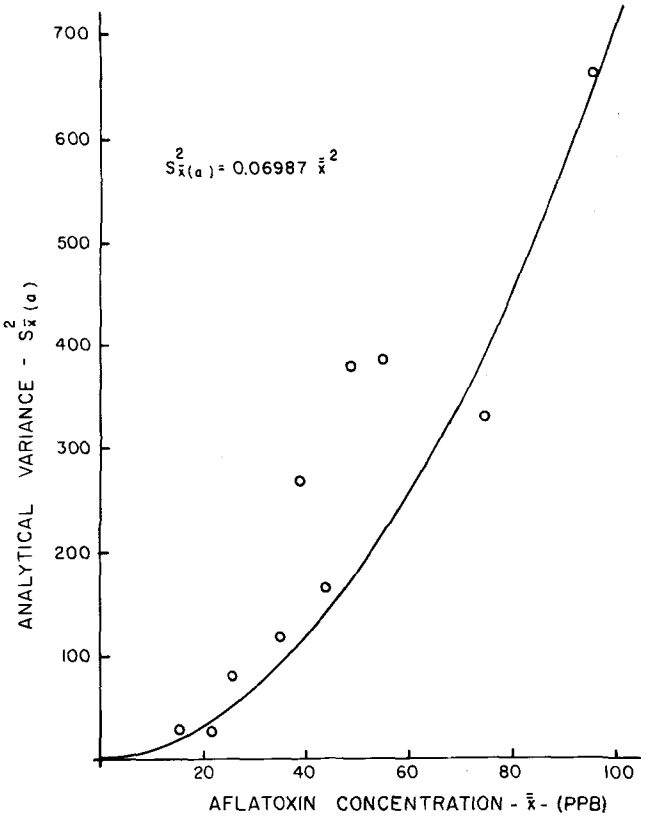

FIG. 2. Relationship between the analytical variance $s^2_{\bar{x}(a)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in ppb.

In this study, both the sampling and subsampling variances were almost completely determined by the laws of probability and thus would not be affected by when the sampling and subsampling operations were performed. On the other hand, analytical variance may be affected by the proficiency of the analyst, the quality of the chemicals and equipment used, and by laboratory conditions. These factors may differ from time to time. To insure that the estimates of analytical variance subtracted from the combined errors were reasonable, analyses to determine $S^2_{\bar{x}(a)}$ were run in combination with analyses to determine $S^2_{\bar{x}(sa)}$, $S^2_{\bar{x}(ca)}$, and $S^2_{\bar{x}(fa)}$.

DISCUSSION

The coefficients c_1, B_1, B_2, and B_3 in equations 6, 13, 14, and 16 were estimated simultaneously in one regression analysis by use of the Statistical Analysis System (11). The correlation coefficient associated with fitting the four equations simultaneously was 0.946.

Analytical Variability

The analytical variance $S^2_{\bar{x}(a)}$ and the average $\bar{\bar{x}}$ for each of the 10 minilots and regression equation 6 are plotted in Figure 2. The results indicate that the analytical variance is a function of the aflatoxin concentration. From the regression analysis the coefficient c_1 was estimated to be 0.06987 with a standard error of 0.01259. Therefore, equation 6 becomes

$$S^2_{\bar{x}(a)} = 0.06987\bar{\bar{x}}^2 \qquad 18.$$

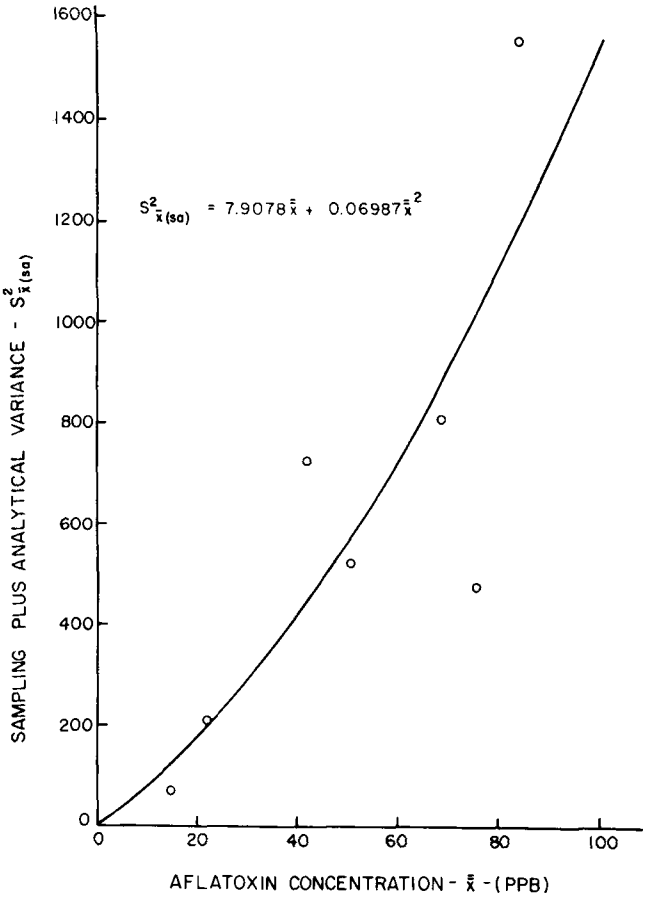

FIG. 3. Relationship between the combined sampling and analytical variance $s^2_{\bar{x}(sa)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in ppb.

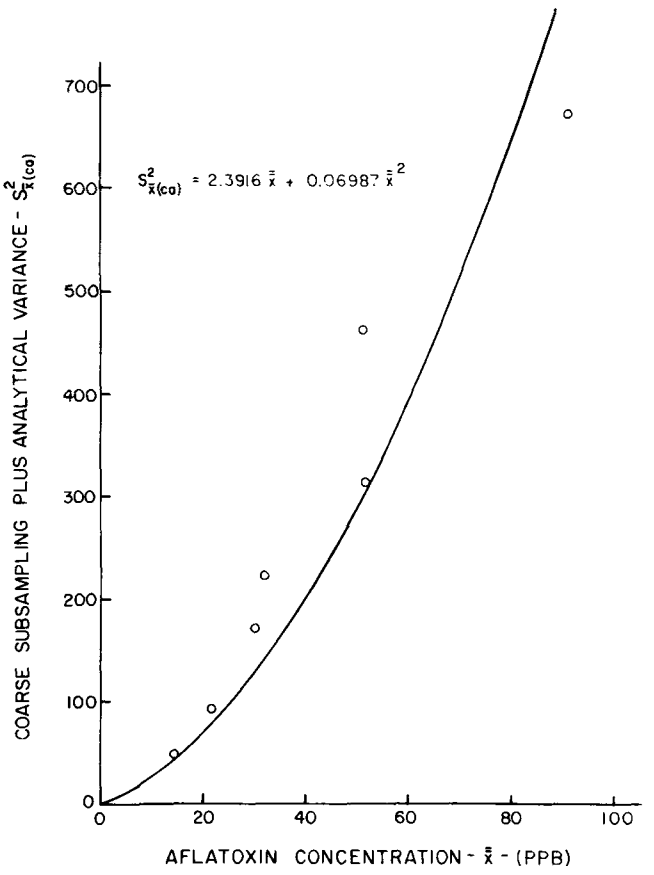

FIG. 4. Relationship between the combined course subsampling and analytical variance $s^2_{\bar{x}(ca)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in ppb.

The coefficient of variation (cv), computed from equation 18, is 26.4% for all mean values μ.

Combined Sampling and Analytical Variance

The combined sampling and analytical variance $S^2_{\bar{x}(sa)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ for each of the 7 minilots and regression equation 13 are plotted in Figure 3. From the regression analysis, B_1 was estimated to be 7.9078 with a standard error of 1.3934. Equation 13 becomes

$$S^2_{\bar{x}(sa)} = 7.9078\,\bar{\bar{x}} + 0.06987\,\bar{\bar{x}}^2 \qquad 19.$$

The first term in equation 19 is the sampling variance.

$$S^2_{\bar{x}(s)} = 7.9078\bar{\bar{x}} \qquad 20.$$

Combined Coarse Subsampling and Analytical Variance

The combined coarse subsampling and analytical variance $S^2_{\bar{x}(ca)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ for each of the 7 minilots and the regression equation 14 are plotted in Figure 4. From the regression analysis, B_2 was estimated to be 2.3916 with a standard error of 1.5382. Equation 14 becomes

$$S^2_{\bar{x}(ca)} = 2.3916\,\bar{\bar{x}} + 0.06987\,\bar{\bar{x}}^2 \qquad 21.$$

The first term in equation 21 represents the coarse subsampling variance.

$$S^2_{\bar{x}(c)} = 2.3916\,\bar{\bar{x}} \qquad 22.$$

Combined Fine Subsampling and Analytical Variance

The combined fine subsampling and analytical variance $S^2_{\bar{x}(fa)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ for each of the 7 minilots and the regression equation 16 are plotted in Figure 5. From the regression analysis, B_3 was estimated to be 0.2503 with a standard error of 0.8627. Equation 16 becomes

$$S^2_{\bar{x}(fa)} = 0.2503\,\bar{\bar{x}} + 0.06987\,\bar{\bar{x}}^2 \qquad 23.$$

The first term of equation 23 represents the fine subsampling variance.

$$S^2_{\bar{x}(f)} = 0.2503\,\bar{\bar{x}} \qquad 24.$$

Application of Results

As shown by the above discussions, the variances for a 500 g sample, 50 g subsample of coarsely ground meal, 50 g subsample of finely ground meal, and one analysis by the CB Method have been developed. The sum of these four components (see equation 2) is the total variance associated with testing shelled corn for aflatoxin. The total variance can be lowered by reduction of one or more of the variance components. One way the variance components can be reduced is to increase the quantity of material analyzed.

The effect of sample size upon the variance of the mean of n items, $\sigma^2_{\bar{x}}\big|_n$, can be demonstrated by use of equation 8. Since the variance among the individual items of a population σ^2 is a fixed parameter, the variance of the mean of n items $\sigma^2_{\bar{x}}\big|_n$ varies inversely with the number of items drawn from the population. By evaluating σ^2 where

$$\sigma^2 = n\,\sigma^2_{\bar{x}}\Big|_n \qquad 25.$$

the variance of the mean of any quantity of material N can be determined by the expression

$$\sigma^2_{\bar{x}}\Big|_N = (n/N)\,\sigma^2_{\bar{x}}\Big|_n \qquad 26.$$

In the case of the present study on corn, n/N is defined as the ratio of sample weights or subsample weights.

From equation 26, the sampling variance for any given sample weight in kg becomes

$$\sigma^2_{\bar{x}(s)}\Big|_{Ns} = (0.5/Ns)\;\sigma^2_{\bar{x}(s)}\Big|_{0.5} \qquad 27.$$

where $\sigma^2_{\bar{x}(s)}\big|_{0.5}$ is given by equation 20. Therefore,

$$\sigma^2_{\bar{x}(s)}\Big|_{Ns} = (0.5/Ns)\;7.9078\,\mu \qquad 28.$$

where Ns is the weight of the sample of shelled corn in kg.

A similar expression can be derived for the coarse subsampling variance for any coarse subsample weight in kg

$$\sigma^2_{\bar{x}(c)}\Big|_{Nc} = (0.05/Nc)\;\sigma^2_{\bar{x}(c)}\Big|_{0.05} \qquad 29.$$

where $\sigma^2_{\bar{x}(c)}\big|_{0.05}$ is given by equation (22) Therefore,

$$\sigma^2_{\bar{x}(c)}\Big|_{Nc} = (0.05/Nc)\;2.3916\,\mu \qquad 30.$$

where Nc is the weight of the coarse ground subsample in kg.

A similar expression can be derived for the fine subsampling variance for any fine subsample weight in kg.

$$\sigma^2_{\bar{x}(f)}\Big|_{Nf} = (0.05/Nf)\;\sigma^2_{\bar{x}(f)}\Big|_{0.05} \qquad 31.$$

where $\sigma^2_{\bar{x}(f)}\big|_{0.05}$ is given by equation 24. Therefore,

$$\sigma^2_{\bar{x}(f)}\Big|_{Nf} = (0.05/Nf)\;0.2503\,\mu \qquad 32.$$

where Nf is the weight of the fine subsample in kg.

The effect of the number of analyses upon the analytical variance is given as follows:

$$\sigma^2_{\bar{x}(a)}\Big|_{Na} = (1/Na)\;\sigma^2_{\bar{x}(a)}\Big|_{1} \qquad 33.$$

where $\sigma^2_{\bar{x}(a)}\big|_1$ is given by equation 18. Therefore,

$$\sigma^2_{\bar{x}(a)}\Big|_{Na} = (1/Na)\;0.06987\,\mu^2 \qquad 34.$$

By addition of equations 28, 30, 32, and 34, the total variance for any given size sample, coarse subsample, fine subsample, and number of analyses can be estimated.

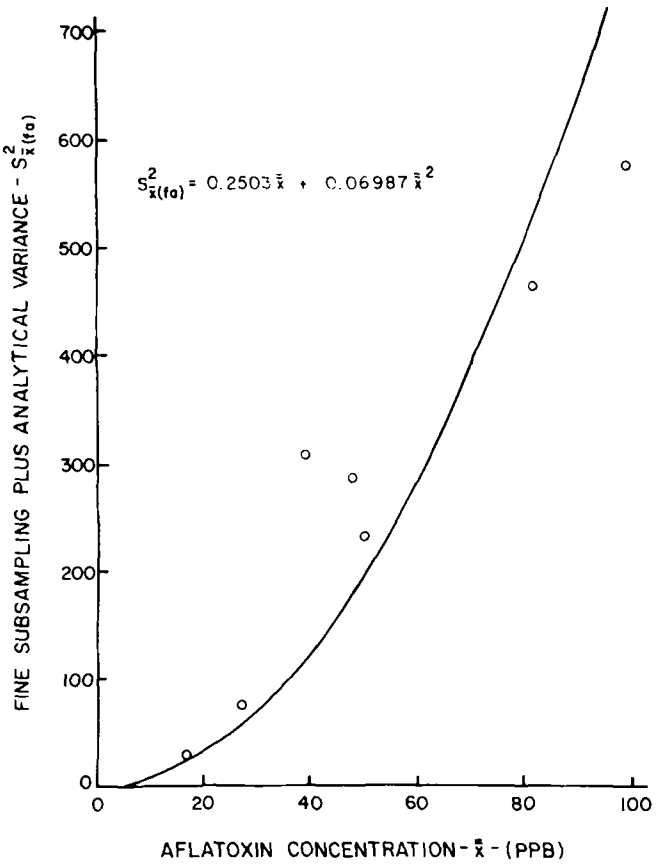

FIG. 5. Relationship between the combined fine subsampling and analytical variance $s^2_{\bar{x}(fa)}$ and the aflatoxin concentration $\bar{\bar{x}}$ in ppb.

$$\sigma^2_{\bar{x}(t)} = [(3.9539/Ns) + (0.1196/Nc) + (0.01252/Nf) + (0.06987/na)\mu]\,\mu \qquad 35.$$

where Ns is the weight of the sample in kg, Nc is the weight of the coarsely ground subsample in kg, Nf is the weight of the finely ground subsample in kg, and Na is the number of analyses. For example, the total variance associated with testing a lot of shelled corn with an aflatoxin concentration of 20 ppb using a 4.54 kg sample, 1 kg subsample of coarse material, 0.05 kg of fine material, and 1 analysis is 52.8. The standard deviation is 7.26 and the CV is 36.3%.

The variances estimated in this study reflect (a) an average kernel weight of 0.328 g, (b) use of a subsampling mill with a 3.2 mm screen to coarsely comminute the sample, (c) use of a Wiley mill with a 1 mm screen to finely grind the coarse material, (d) use of the CB Method for analysis, and (e) use of densitometric equipment to quantify intensities of spots in thin layer chromatographic analyses.

The variance estimates are also based on the assumption that the negative binomial function described the distribution of the aflatoxin-contaminated kernels in the corn.

Application of these variance estimates to commercial lots would depend upon the assumption that the distribution according to aflatoxin concentration of contaminated kernels in the minilots was similar to the distribution in commercial lots with the same aflatoxin concentration.

Sampling and subsampling variance estimates would probably be consistent from one laboratory to another if similar grinding equipment were used, but analytical variability may differ among laboratories (12,13). The variance relationships presented in this study should indicate the major sources of error in testing corn for aflatoxin and

provide insights concerning ways to reduce the total variability.

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[Received November 17, 1978]