SAMPLING GRANULAR FOODSTUFFS FOR AFLATOXIN (Note a.)

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Abstract – Methodology is described for the design and evaluation of testing programs to estimate aflatoxin concentrations in lots of granular foodstuffs. Use of operating characteristic curves and of the prior distribution of lot concentrations for comparing and evaluating processor and consumer risks related to testing programs are demonstrated. Operating characteristic curves, computed from a system of equations that accounts for errors in sampling, subsampling, and analysis are developed for the 1976 peanut aflatoxin testing program in the United States. Estimates are given of aflatoxin concentration in lots accepted and rejected by the testing program.

INTRODUCTION

Aflatoxin is found in corn, cottonseed, peanuts, pistachio nuts, and other agricultural commodities. Estimates of the aflatoxin concentration in lots of granular material are based upon analyses of samples taken from these lots. (The term granular refers to whole intact commodities, such as those mentioned above, and not to ground material.) If the estimated lot concentration is greater than an established guideline or tolerance, then the product is diverted from food channels. However, it is difficult to accurately estimate the lot concentration due to the large variability associated with replicated test results on a contaminated product.

Typical steps taken to estimate the aflatoxin concentration in a lot of granular material are shown in Fig. 1. A random sample drawn from the lot is comminuted in a grinder or mill to reduce the particle size and increase homogeneity of the material, a subsample of comminuted material taken from the sample is chemically analyzed for aflatoxin. Thus the total error associated with aflatoxin test results is the sum of at least three main components: errors in sampling, subsampling, and analysis.
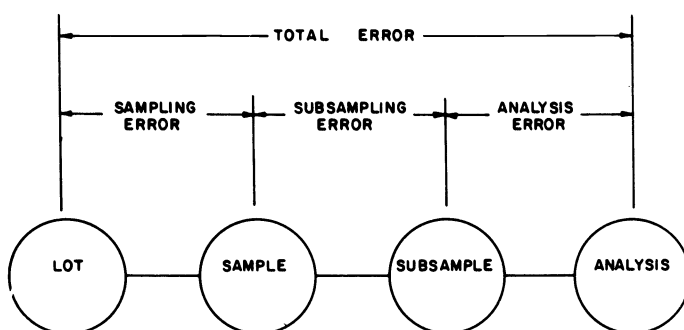

Fig. 1. Typical steps employed to estimate the aflatoxin concentration $\bar{x}$ and the associated variance components,
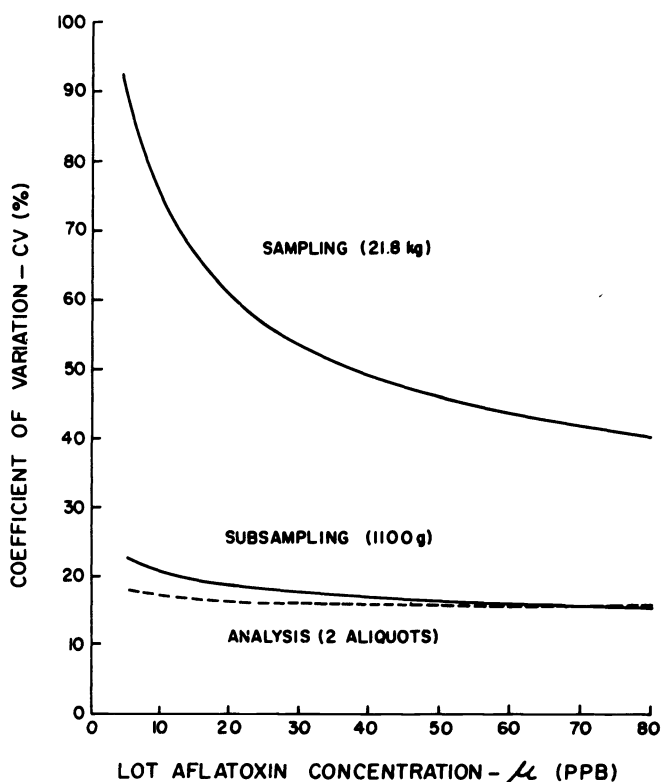
Fig. 2. Coefficient of variation associated with the sampling, subsampling, and
analytical steps of the peanut aflatoxin testing program.

Studies (Ref. 1,2) on peanuts and cottonseed indicated for the size samples now used
(less than 22 kg) sampling generally is the largest source of error. That error is
large because aflatoxin is found in a small percentage of the kernels less than 0.1%,
but the level of contamination on a single kernel may be as high as 1,000,000 parts
per billion (ppb) (Ref. 3). Because of this wide range in aflatoxin concentration
among individual particles in a contaminated lot, variations among replicated samples
tend to be large.

The same type of extreme distribution of contaminated particles is assumed to exist
in the ground sample so that for a given aflatoxin concentration the variability is
the same among the comminuted particles as among the kernels before comminution.
Because comminution reduces the size and increases the number of particles, the
error usually is less in subsampling than in sampling.

Chemical analysis of subsamples is a complex process involving many steps such as
extraction, concentration, dilution, and plating. Each step provides possible
sources of error and contributes to the overall variability associated with
chemical assay methods.

Figure 2 illustrates the magnitude of the errors associated with sampling, sub-
sampling, and analysis steps of the aflatoxin testing program for peanuts. At
a lot concentration of 20 ppb the coefficient of variation (CV) was about 60% for a
21.8 kg (48 pounds) sample, 18% for a 1100 g subsample, and 16% for the analysis of
two aliquots (Ref. 4). The total error for the above system for a lot concentration
of 20 ppb was estimated to be 80%. The above errors were estimated empirically and
would differ for other commodities (Ref. 1,2).

Because of the large errors associated with an aflatoxin testing program, analyses
of samples from a "good" lot may indicate that the lot is "bad" (processor's risk)
and at other times analyses of samples from a "bad" lot may indicate that the lot
is "good" (consumer's risk). Thus, with a given aflatoxin testing program there
are associated a certain consumer's risk, processor's risk, and cost. To maintain
effective quality control, the risks and costs associated with a testing program
must be evaluated. On the basis of these evaluations, a testing program can be

designed or selected to provide a high level of protection for both the consumer and the processor at the lowest possible cost.

The objective of this paper is to discuss a method that has been developed to evaluate the risks associated with testing granular material for aflatoxin.

MATHEMATICAL AND THEORETICAL CONSIDERATIONS

Operating characteristic curve

As a consequence of a testing program, a lot of granular material is judged acceptable or unacceptable depending upon analyses of samples drawn from the lot.

A sample may be termed "bad" when the aflatoxin test result $\bar{x}$ is above some predefined success level $\bar{x}_c$ and "good" when $\bar{x} \leq \bar{x}_c$. Lots with an aflatoxin concentration μ will be accepted as good with a certain probability $P(\mu) = \text{Prob } (\bar{x} \leq \bar{x}_c | \mu)$. A plot of the acceptance probability $P(\mu)$ versus lot concentration μ is called an operating characteristic (OC) curve, and Fig. 3 depicts its general shape. As μ approaches zero $P(\mu)$ approaches 1, and as μ becomes large $P(\mu)$ approaches zero. The shape of the OC curve is uniquely defined for a particular testing program with designated values of sample size, subsample size, number of analyses, and the definition of good and bad sample quality $\bar{x}_c$.

For a given testing program, the OC curve indicates the magnitudes of the consumer and processor risks. When μ_c is defined as the maximum concentration of aflatoxin acceptable, lots with $\mu > \mu_c$ are bad and lots with $\mu \leq \mu_c$ are good. In Fig. 3, the area beneath the OC curve for $\mu > \mu_c$ represents the consumer risk (bad lots accepted) while the area above the OC curve for $\mu \leq \mu_c$ represents the processor risk (good lots rejected) for a particular testing program.
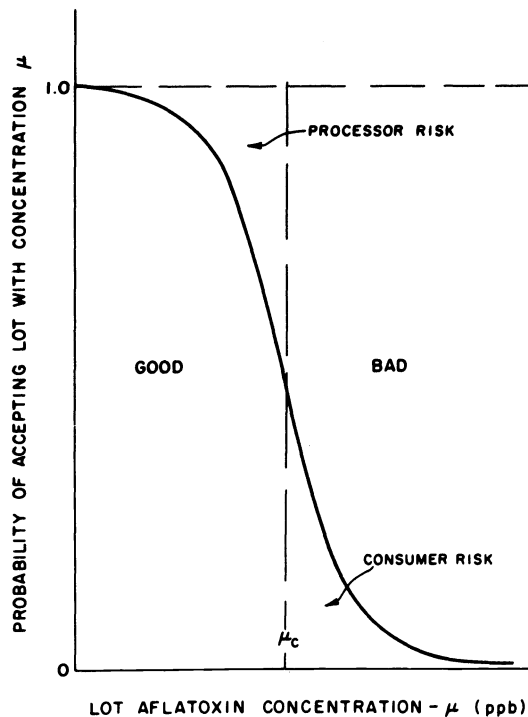

Fig. 3. Typical operating characteristic curve for evaluating aflatoxin testing programs.

Prior distribution

The areas above and below the OC curve, which are related to the consumer and processor risks, can be quantified if a prior distribution is estimated. The prior distribution is defined as the distribution of lot aflatoxin concentrations. The prior distribution can be approximated by assuming that the distribution of lot concentrations is the same as the observed distribution of test results from previous analyses of a large number of lots. The total number of lots having a specific aflatoxin concentration μ is

L f(μ) where L is the total number of lots and f(μ) is the decimal fraction of L lots with concentration μ. The number of lots accepted by a testing program at a given concentration μ is the product of the acceptance probability P(μ) and the number of lots with the given concentration L f(μ).

$$L_a(\mu) = L\ f(\mu)\ P(\mu). \tag{1}$$

Figure 4 illustrates the OC curve P(μ), the prior distribution L f(μ), and the number of lots accepted $L_a(\mu)$ at various lot concentrations. For a given testing program, the total number of lots accepted may be computed by summing equation 1 across all lot concentrations μ.

$$L_a = \sum_{\mu=0}^{\infty} L\ f(\mu)\ P(\mu). \tag{2}$$

The number of good lots ($\mu \leq \mu_c$) accepted is

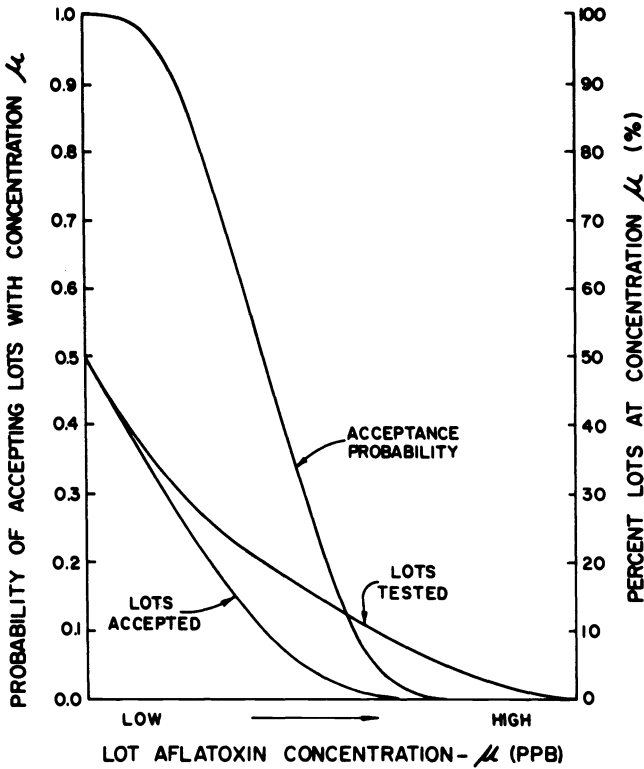$$GL_a = \sum_{\mu=0}^{\mu=\mu_c} L\ f(\mu)\ P(\mu). \tag{3}$$

Fig. 4. Operating characteristic curve, prior distribution of lot concentra-
tions and the number of lots accepted at various lot concentrations.

The number of bad lots ($\mu > \mu_c$) accepted is

$$BL_a = \sum_{\mu=\mu_c + \Delta}^{\infty} L \cdot f(\mu) \cdot P(\mu) \tag{4}$$

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

$$GL_r = \left[\sum_{\mu=0}^{\mu_c} L\ f(\mu) \right] - GL_a \tag{5}$$

where the term in the brackets is the total number of good lots to be inspected by the testing program. For a given testing program, the number of bad lots accepted, BL_a, indcates the consumer's risk and while the number of good lots rejected, GL_r, indicates the processor's risk. The average aflatoxin concentration in all lots accepted by a

testing program is

$$AA = \left[\sum_{\mu=o}^{\infty} \mu \ L \ f(\mu) \ P(\mu) \right] / L_a \qquad (6)$$

Theoretical Model

To compute the acceptance probability $P(\mu)$, the distribution of aflatoxin test results $\bar{x}$ must be determined as a function of lot concentration μ, sample size N_s, subsample size N_{ss}, and number of analyses N_a.

Three distributions should be considered in describing the variability of test results $\bar{x}$ about the lot concentration μ: (a) distribution of sample concentrations $\bar{x}_s$ about the lot concentration μ, (b) distribution of subsample concentrations $\bar{x}_{ss}$, about the sample concentration μ_s, and (c) distribution of analytical determinations $\bar{x}$ about the subsample concentration μ_{ss}. Previous studies on peanuts and cottonseed (Ref. 5,6) have indicated that the sample and subsample distributions can be simulated best with skewed type probability distribution functions while the analytical distribution tends to be more normal in nature.

EVALUATION OF A PEANUT TESTING PROGRAM

The aflatoxin testing program used by the U. S. peanut industry in 1976 was selected on the basis of evaluations by the techniques described in the previous section. Figure 5 is a diagram of the testing program. A 21.8 kg (48 pound) sample of kernels is comminuted in a subsampling mill and a 1100-g subsample is extracted for aflatoxin analysis. Two analyses, identified as 1A and 1B, are made on the extract. If the average of 1A and 1B is 16 parts per billion (ppb) aflatoxin or less, the lot is accepted; if the average is more than 75 ppb the lot is rejected. Otherwise, a second 21.8 kg sample is processed and two more analyses are made (2A and 2B). If the average of 1A, 1B, 2A, and 2B is 22 ppb or less, the lot is accepted; if the average is greater than 38 ppb, the lot is rejected. Otherwise, a third 21.8 kg sample is processed and two more analyses are made (3A and 3B). If the average of 1A, 1B, 2A, 2B, 3A, and 3B is 25 ppb or less, the lot is accepted; if the average is greater than 25 ppb, the lot is rejected.
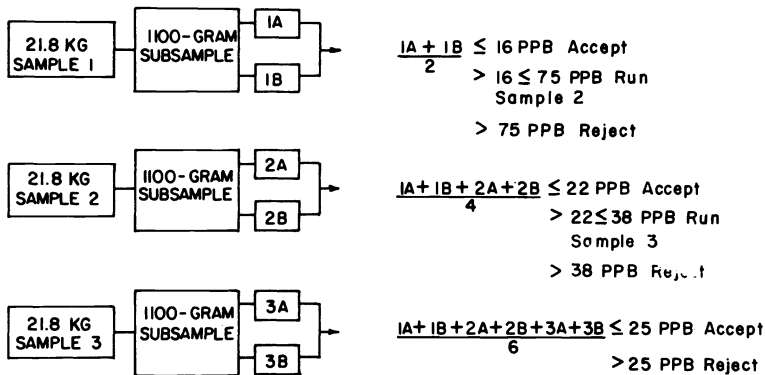

Fig. 5. Schematic of the 1976 peanut aflatoxin testing program.

The negative binomial distribution function adequately describes the distribution of peanut sample aflatoxin concentrations $\bar{x}_s$ about the lot aflatoxin concentration μ (Ref. 5).

$$F_s(N_s\bar{x}_s) = \sum_{r=o}^{N_s\bar{x}_s} \left[\Gamma(r+N_sK_s)/(r! \ \Gamma(N_sK_s))) \right.$$

$$\left. (K_s/(K_s + \mu))^{N_sK_s} \ (\mu/(K_s + \mu))^r \right] \qquad (7)$$

where Γ is the gamma function, N_s is the sample size in number of kernels, and K_s is the "shape parameter" determined by the aflatoxin concentration in the lot.

The distribution of subsample concentrations $\bar{x}_{ss}$ about the sample concentration μ_s was also assumed to be negative binomial. This assumption was made since the distribution of aflatoxin-contaminated particles in the comminuted sample is probably similar to the distribution of contaminated kernels in the sample before comminution.

$$F_{ss}(N_{ss}\bar{x}_{ss}) = \sum_{r=0}^{N_{ss}\bar{x}_{ss}} \left[(\Gamma(r + N_{ss}K_{ss})/(r!\, \Gamma(N_{ss}K_{ss}))) \right.$$

$$\left. (K_{ss}/(K_{ss} + \mu_s))^{N_{ss}K_{ss}} (\mu_s/(K_{ss} + \mu_s))^r \right] \qquad (8)$$

where N_{ss} is the number of comminuted particles in the subsample and K_{ss} is the "shape parameter" determined by the aflatoxin concentration in the sample. A study of subsampling variability (Ref. 1) indicated that the subsampling variance is large compared to μ_s which is characteristic of skewed distributions such as the negative binomial function.

The normal distribution was chosen to simulate the distribution of analytical results $\bar{x}$ about the subsample concentration μ_{ss}. The studies indicated the normal distribution can accurately simulate the distribution of analytical results $\bar{x}$ over a wide range of subsample concentrations (Ref. 7).

$$F_a(\bar{x}) = \int_0^{\bar{x}} (1/((2\pi)^{0.5}\, \sigma_x))\, \mathrm{Exp}\, (-(\bar{x} - \mu_{ss})^2 / \qquad (9)$$

$$(2\sigma_x^2))\, d\bar{x}.$$

Monte Carlo Solution
The acceptance probabilitites for the present peanut aflatoxin testing program were determined by Monte Carlo procedures (Ref. 8). Monte Carlo was selected because it provided a means to account for conditional probabilities that arise from the use of multiple samples, subsamples, and/or analyses in a testing program.

With the Monte Carlo method, a random number generator (Ref. 9,10) simulates the random selection of a sample, subsample, or analysis. For simulation of the drawing of a sample from a contaminated lot with aflatoxin concentration μ a random number, uniformly distributed between 0 and 1, is generated. This number is taken as the value of $F_s(N_s\bar{x}_s)$ in equation 7 for which the corresponding value of $N_s\bar{x}_s$ is determined. Then sample size N_s is specified, and the sample concentration x_s is computed.

The above sample is then treated as a new population independent of the lot. In equation 8, μ_s takes on the value of $\bar{x}_s$ computed above. For simulation of the drawing of a subsample from the above sample another random number, uniformly distributed between 0 and 1, is generated. This number is taken as the value of $F_s(N_{ss}\bar{x})$ in equation 8 for which the corresponding value of $N_{ss}\bar{x}$ is determined. The subsample size N_{ss} is specified, and the subsample concentration $\bar{x}_{ss}$ is computed.

The above subsample is then treated as a new population independent of the sample. In equation 9, μ_{ss} takes on the value of $\bar{x}_{ss}$ obtained above. For simulation of a chemical analysis on the above subsample with aflatoxin concentration $\bar{x}_{ss}$ another random number, uniformly distributed between 0 and 1, is generated. In equation 9 this number is taken as the value of $F_a(\bar{x})$ for which the corresponding value of $\bar{x}$ is determined.

A computer program was written to determine the probability of accepting a lot with the specified aflatoxin concentration μ. The following values were specified: N_s = 21.8 kg (48 pounds), N_{ss} = 1100-g, and N_a = 2. Figure 6 is a flow chart describing the computer program. Analytical results simulating the testing of 2000 lots with the same μ value were generated. The acceptance probability was then computed by dividing the number of lots accepted by 2000. The above procedure was repeated for various levels of lot concentration μ. Figure 7 is a plot of the acceptance probabilities as a function of lot concentration μ. The OC curve indicates that all lots with 10 ppb or less aflatoxin would be accepted by the testing program, while all lots in excess of 70 ppb aflatoxin would be rejected.

The acceptance probabilities for the OC curve shown in Fig. 7 were transformed into lots accepted and rejected by use of the 1974 prior lot distribution; the 1974 crop was typical of previous crops years. The distribution, shown in Table 1, had an average aflatoxin concentration of 5 ppb. It is estimated that of the 20,710 lots tested, 20,352 (98.27%) had 25 ppb or less aflatoxin while 358 (1.73%) had concentrations in excess of 25 ppb. Table 1 shows that the number of lots accepted and rejected at
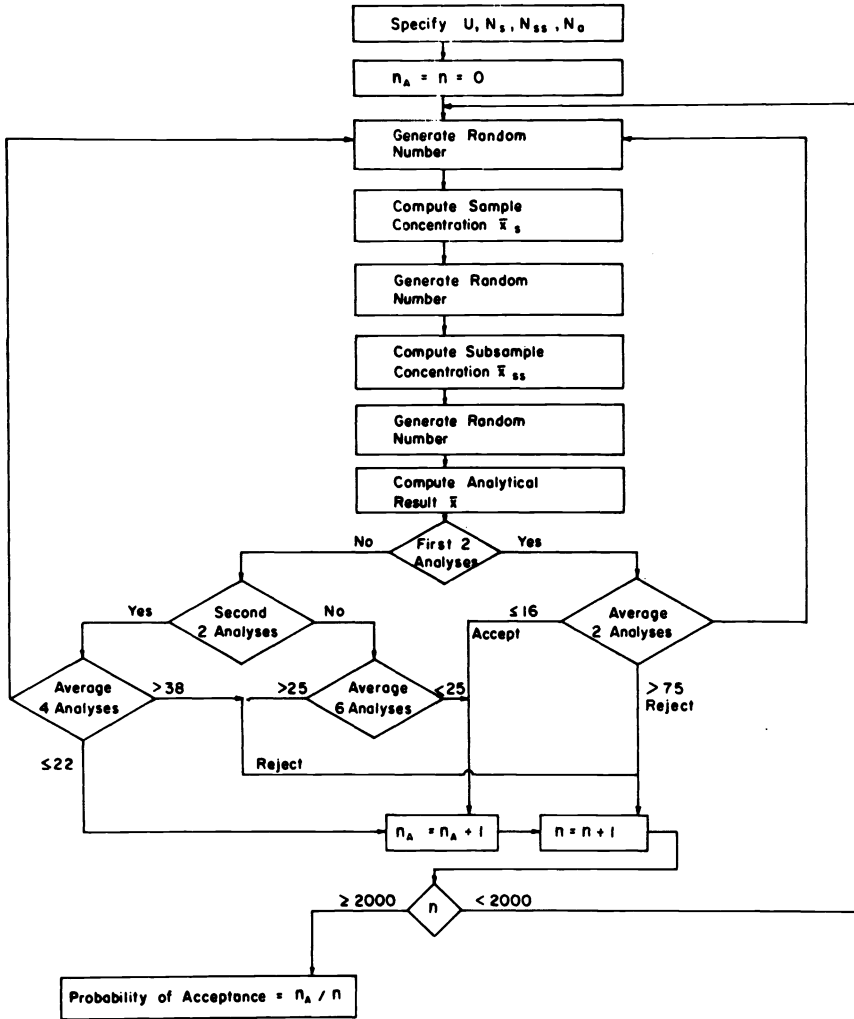
Fig. 6. Flow chart describing the computer model and Monte Carlo solution technique.
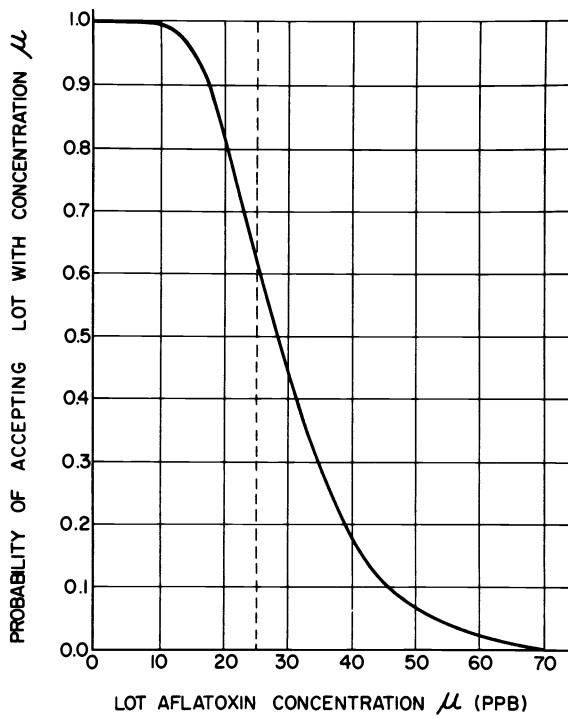
Fig. 7. Operating characteristic curve of the 1976 peanut aflatoxin testing
        program.

various aflatoxin concentrations.

TABLE 1. Number of lots tested, accepted, and rejected by the 1976 peanut aflatoxin testing program when used on a crop similar to the 1974 peanut crop.

Lot Concentration	Number Lots Tested	Number Lots Accepted	Number Lots Rejected
0	4467	4467	0
1	2884	2884	0
2	2193	2193	0
3	1751	1751	0
4	1431	1431	0
5	1187	1187	0
6	993	993	0
7	708	708	0
9	601	601	0
10	512	512	0
11 - 12	811	809	2
13 - 14	596	588	8
15 - 16	440	426	14
17 - 18	327	302	25
19 - 20	243	209	34
21 - 25	372	271	101
26 - 30	181	96	85
31 - 35	89	22	67
36 - 40	44	9	35
41 - 45	22	2	20
46 - 50	11	1	10
51 - 60	8	0	8
61 - 70	2	0	2
> 70	1	0	1

Of the 20,710 lots tested, 20,298 (98.01%) were accepted and 412 (1.99%) were rejected. In the 20,298 lots accepted aflatoxin levels were 25 ppb or less in 20,618 (99.36%) and exceeded 25 ppb in 130 (0.64%). Of the accepted lots, aflatoxin concentration exceeded 40 ppb in only 3 and exceeded 50 ppb in no lots. Of the 412 lots rejected, 184 (44.66%) had 25 ppb or less aflatoxin and 228 (55.34%) had concentrations in excess of 25 ppb. No lots with 10 ppb or less aflatoxin were rejected by the testing program.

DISCUSSION

The procedure discussed in this paper for estimating the consumer and processor risks provides an objective method to evaluate and compare aflatoxin testing programs for granular foodstuffs. The basic concepts concerning the use of the OC curve and prior distribution might be applied to the control of other mycotoxins. The accuracy of the evaluations depends upon the validity of the mathematical models and the correct choice of model parameters.

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