Evaluation of Cottonseed Aflatoxin[1] Testing Programs

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ABSTRACT AND SUMMARY

A computer model was developed to simulate cottonseed aflatoxin testing programs. By use of the model, probabilities of obtaining certain aflatoxin test results for various lot concentrations and sample sizes were determined. Also, the effects of sample size and the definition of good and bad sample quality on the probability of lot acceptance were demonstrated.

INTRODUCTION

The concentration of aflatoxin in cottonseed lots is estimated by analyzing samples taken from each lot. The estimations are difficult to make because of the large variability associated with replicated test results on the contaminated lot (1,2). Typical steps taken to estimate the aflatoxin concentration in cottonseed lots are shown in Figure 1. A random sample of cottonseed is drawn from the lot, the sample is dehulled, the kernels are comminuted in a mill, and a subsample is analyzed for aflatoxin. Thus, the total error associated with aflatoxin test results have at least three error components: sampling, subsampling, and analysis.

Figure 2 illustrates the magnitudes of the error components in the cottonseed aflatoxin testing program. At a lot concentration of 20 parts per billion (PPB), the coefficient of variation (CV) is about 95% for a 2270-g (5-lb) sample of dehulled kernels, 15% for a 200-g subsample, and 8% for the analysis of one aliquot.

Because of the large errors associated with testing cottonseed for aflatoxin, 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 certain risks to both the consumer and processor. If an effective quality control program is to be developed, these risks associated with the testing program must be evaluated. A testing program could then be designed or selected to provide a high level of protection for both the consumer and the processor at the lowest possible cost.

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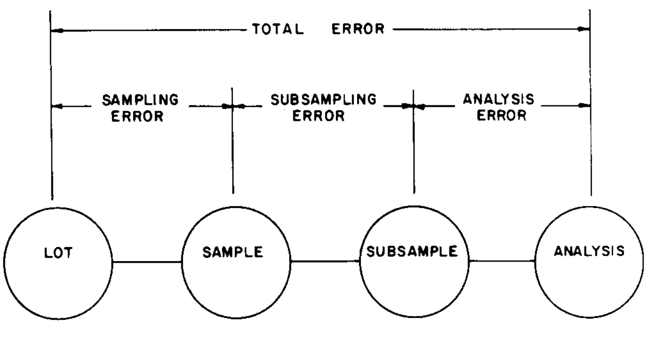

FIG. 1. Typical steps employed to estimate the aflatoxin concentration $\bar{x}$ and the associated variance components.

The objectives of this study were to provide basic information necessary to evaluate cottonseed aflatoxin testing programs and to demonstrate the effects of sample size and the definition of good and bad sample quality on the consumer's and processor's risks.

PROCEDURE

The procedure developed to evaluate cottonseed aflatoxin testing programs is similar to that used to evaluate peanut aflatoxin testing programs. A detailed discussion of the latter is provided in previous publications (3,4).

Operating Characteristic Curve

As a consequence of a testing program, a cottonseed lot is judged acceptable or unacceptable on the basis of the 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 $\bar{x} < \bar{x}_c$. Lots with aflatoxin concentrations 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 Figure 3 depicts its general shape. As μ approches 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, the definition of good and bad sample quality $\bar{x}_c$, and the associated probability distribution.

For a given testing program, the OC curve indicates the magnitudes of both the consumer's and processor's risks. When μ_c is defined as the maximum concentration of aflatoxin acceptable, lots with $\mu > \mu_c$ are bad, and lots

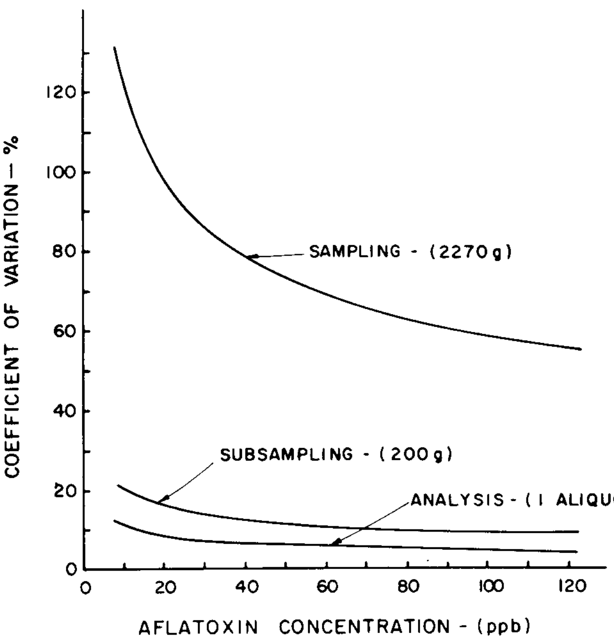

FIG. 2. Coefficient of variation associated with the sampling, subsampling, and analytical stages of the cottonseed aflatoxin testing program.

with $\mu \leq \mu_c$ are good. In Figure 3, the area beneath the OC curve for $\mu > \mu_c$ is proportional to the consumer's risk (bad lots accepted), whereas the area above the OC curve for $\mu \leq \mu_c$ is proportional to the processor's risk (good lots rejected) for a particular testing program.

Theoretical Model

For computation of 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 may be used to describe 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 concentration $\bar{x}_{ss}$ about the sample concentration μ_s, and (c) distribution of analytical determinations $\bar{x}_a$ about the subsample concentration μ_{ss}. Previous studies on peanuts and cottonseed (1,2,5) have indicated that the sample and subsample distributions can be simulated best with a skewed-type probability distribution function, whereas the analytical distribution tends to be more nearly normal.

The negative binomial distribution function was assumed to adequately describe the distribution of cottonseed sample aflatoxin concentrations $\bar{x}_s$ about the lot concentration μ.

$$F_s(N_s\bar{x}_s) = \sum_{r=0}^{N_s\bar{x}_s} \left[\frac{\Gamma(r+N_sK_s)}{r!\Gamma(N_sK_s)} \left(\frac{K_s}{K_s+\mu}\right)^{N_sK_s} \left(\frac{\mu}{K_s+\mu}\right)^{r} \right] \quad \text{(I)}$$

where Γ is the gamma function, N_s is the sample size in grams of cottonseed 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 a 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.

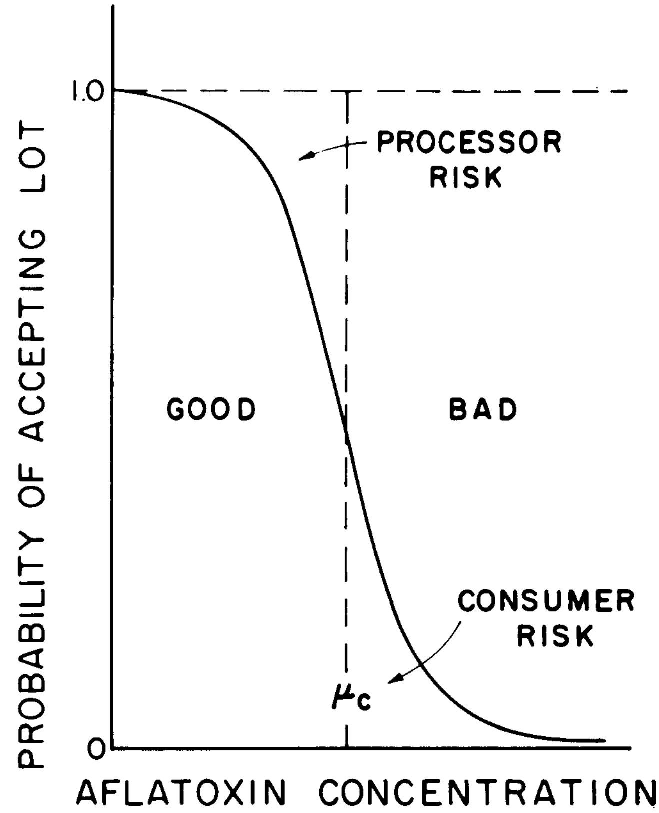

FIG. 3. Typical operating characteristic curve for evaluating aflatoxin testing programs.

$$F_{ss}(N_{ss}\bar{x}_{ss}) = \sum_{r=0}^{N_{ss}K_{ss}} \left[\frac{\Gamma(r+N_{ss}K_{ss})}{r'!\Gamma(N_{ss}K_{ss})} \left(\frac{K_{ss}}{K_{ss}+\mu_s}\right)^{N_{ss}K_{ss}} \left(\frac{\mu_s}{K_{ss}+\mu_s}\right)^{r} \right] \quad \text{(II)}$$

TABLE I

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = .908 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot—μ (ppb)									
	2	5	10	15	20	30	40	60	80	100
0	304	108	028	009	003	001	000	000	000	000
1	785	605	419	301	221	124	073	027	010	004
2	834	677	501	381	294	181	114	048	021	010
3	863	722	555	436	347	225	149	068	033	016
5	898	780	630	515	424	293	206	105	055	029
7	920	818	681	572	483	348	254	138	077	044
10	942	858	738	637	551	416	316	185	109	066
15	963	900	802	714	635	504	400	254	162	104
20	975	927	845	768	697	573	469	315	212	142
25	983	945	877	810	745	629	528	370	259	181
30	988	958	901	842	784	675	578	420	304	218
35	991	967	919	867	815	715	622	466	346	255
40	994	974	934	888	841	748	661	508	385	291
50	997	984	954	919	882	803	725	580	458	358
60	998	990	968	941	911	844	775	642	522	420
80	999	996	984	968	948	901	848	738	629	530
100	1000	998	992	982	969	936	897	807	713	620
120		999	996	990	981	958	929	858	778	695
140		1000	998	994	988	973	951	895	828	755
160			999	996	993	982	966	922	867	804
200			1000	999	997	992	984	957	920	875
250				1000	999	997	993	980	958	929
300					1000	999	997	990	978	960

[a]Probabilities reflect a 200 g subsample and one analysis.

TABLE II

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = 2.227 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot—μ (ppb)									
	2	5	10	15	20	30	40	60	80	100
0	052	004	000	000	000	000	000			
1	653	388	178	087	044	012	003	000	000	
2	751	508	275	154	088	030	010	001	000	
3	809	588	351	213	130	505	020	003	001	
5	877	697	459	315	211	096	044	009	002	000
7	917	769	559	401	286	144	073	019	005	001
10	951	841	661	507	385	218	122	037	011	004
15	978	911	775	642	522	335	210	079	029	011
20	989	948	849	738	629	441	300	131	055	023
25	995	969	897	807	713	534	386	189	088	040
30	997	981	929	858	778	613	466	251	127	062
35	999	988	951	895	828	681	539	313	170	089
40	999	993	966	923	867	737	603	374	216	120
50	1000	997	984	957	921	823	711	489	313	190
60		999	992	977	953	882	792	591	409	269
80		1000	998	993	983	949	895	748	582	430
100			1000	998	994	978	948	850	718	577
120				999	998	991	975	914	817	697
140				1000	999	996	988	951	884	790
160					1000	998	994	973	928	858
260						1000	999	992	973	938
250							1000	998	993	980
300								1000	998	994

[a]Probabilities reflect a 200 g subsample and one analysis.

TABLE III

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = 4.54 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot—μ (ppb)									
	2	5	10	20	30	40	50	60	80	100
0	003	000	000	000	000	000				
1	537	221	055	014	004	000				
2	690	367	126	044	015	002	000			
3	781	480	201	082	034	006	001			
5	884	644	342	172	085	020	005	000		
7	935	754	466	268	149	043	012	001		
10	972	856	616	407	257	095	033	004	000	
15	992	941	783	601	437	209	092	015	002	000
20	998	975	880	741	592	339	176	040	008	002
25	999	990	934	836	713	466	275	079	020	005
30	1000	996	964	898	803	581	379	132	039	010
35		998	981	937	867	678	480	196	067	021
40		999	990	962	911	757	573	268	104	036
50		1000	997	986	962	867	726	420	201	084
60			999	995	984	931	833	564	318	156
80			1000	999	997	983	944	783	559	349
100				1000	1000	996	983	905	749	554
120						999	995	962	871	724
140						1000	999	986	939	843
160							1000	995	973	916
200								999	995	980
250								1000	1000	997
300										1000

[a]Probabilities reflect a 200 g subsample and one analysis.

where N_{ss} is the subsample size in grams of comminuted particles in the subsample, and K_{ss} is the "shape parameter" determined by the aflatoxin concentration in the sample.

The normal distribution was chosen to simulate the distribution of analytical results $\bar{x}_a$ about the subsample concentration μ_{ss}. The normal distribution was chosen because studies with peanuts indicated that the normal distribution accurately simulates the distribution of analytical results $\bar{x}_a$ over a wide range of subsample concentrations (6).

$$F(\bar{x}_a) = \int_0^{\bar{x}_a} (1/\sqrt{2\pi\sigma_a^2})\, \mathrm{EXP}\left[-(\bar{x}_a-\mu_{ss})^2/2\sigma_a^2\right] d\bar{x}_a \quad \text{(III)}$$

where σ_a^2 is the variance among single analytical determinations of a subsample with concentration μ_{ss}, and N_a is the number of equal portions of extract taken from the blender and analyzed by the Velasco method (7).

Sample size N_s, subsample size N_{ss}, and the number of analyses N_a are specified by the aflatoxin testing procedure. The parameters K_s, K_{ss}, and σ_a^2 for the above system of equations must be estimated. The shape parameter K for the negative binomial distribution function is defined as

$$K = \mu^2/(\sigma^2-\mu) \quad \text{(IV)}$$

where σ^2 is the variance among individual members of a population, and μ is the concentration of the population.

TABLE IV

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = 9.08 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot–μ (ppb)									
	2	5	10	20	30	40	50	60	80	100
0	000	000	000	000						
1	414	089	007	001	000					
2	639	229	034	005	001					
3	776	370	048	016	003	000				
5	912	604	217	065	018	001				
7	965	762	371	146	051	005	000			
10	991	895	583	304	139	023	003	000		
15	999	975	816	572	350	099	022	001		
20	1000	994	927	769	566	233	075	005	000	
25		999	973	885	735	395	165	018	001	
30		1000	991	947	851	558	286	046	005	000
35			997	976	920	695	421	094	013	001
40			999	990	959	800	552	162	030	004
50			1000	998	990	924	764	345	099	021
60				1000	998	974	891	546	221	064
80					1000	998	983	839	542	261
100						1000	998	958	800	539
120							1000	991	932	771
140								999	981	907
160								1000	995	968
200									1000	997
250										1000
300										

[a]Probabilities reflect a 200 g subsample and one analysis.

TABLE V

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = 18.16 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot–μ (ppb)									
	2	5	10	20	30	40	50	60	80	100
0	000	000								
1	291	019	000							
2	601	111	004	000						
3	794	259	018	001	000					
5	951	575	108	012	001	00				
7	989	793	273	055	008	000				
10	999	942	559	200	052	002	000			
15	1000	994	869	552	257	029	002			
20		1000	971	817	547	131	018	000		
25			995	940	779	321	073	001		
30			999	984	910	541	190	007	000	
35			1000	996	968	730	356	027	001	
40				999	990	859	537	073	004	000
50				1000	999	971	820	263	031	002
60					1000	996	949	533	125	014
80						1000	998	904	530	168
100							1000	990	866	528
120								1000	998	963
140									1000	994
200										1000
250										
300										

[a]Probabilities reflect a 200 g subsample and one analysis.

The sampling, subsampling, and analytical variances were estimated for cottonseed in a previous study (2).

$$\sigma_s^2 = 108.1126\,\mu^{1.3434} - 5.3994\,\mu^{1.3508} - \mu^{1.2421}; \qquad \text{(V)}$$

$$\sigma_{ss}^2 = 35.9600\,\mu^{1.3508}; \qquad \text{(VI)}$$

$$\sigma_a^2 = 0.0666\,\mu^{1.2421} \qquad \text{(VII)}$$

Equations V and VI, substituted into Equation IV, provide estimates of K_s and K_{ss}, respectively. The expression for the analytical variance in Equation VII is substituted directly into Equation III.

The system of equations can be solved by the Monte Carlo technique. With this technique a random number generator is used to simulate the drawing of a sample from a lot, the drawing of a subsample from a sample, and the analysis of a subsample. The Monte Carlo technique is described in detail in a previous publication (8).

RESULTS

The theoretical equations described in the previous section were used to compute the cumulative distribution of sample concentrations for various lot concentrations μ and sample sizes N_s as shown in Tables I through VI. The distributions are for one analysis of a 200 g subsample. The cumulative distribution gives the probability of obtaining a test result $\bar{x}$ or less for a given lot concentration

TABLE VI

Probability (x1000) of Obtaining a Test Result $\bar{x}$ or Less from a Lot of Cottonseed with Concentration μ and Sample Size N_s = 27.24 kg[a]

Test result $\bar{x}$ (ppb)	Aflatoxin concentration in lot–μ (ppb)									
	2	5	10	20	30	40	50	60	80	100
0	000	000								
1	222	005								
2	584	061	000							
3	814	197	005	000						
5	972	562	060	003	000					
7	996	821	216	024	002					
10	1000	966	549	143	022	000				
15		999	903	543	202	010	000			
20		1000	987	851	539	082	005			
25			999	967	812	273	037			
30			1000	994	943	534	136	001		
35				999	986	759	315	009		
40				1000	997	897	531	036	000	
50					1000	988	858	212	001	000
60						999	974	527	078	004
80						1000	1000	939	525	116
100								998	905	524
120								1000	992	875
140									1000	983
160										999
200										1000
250										
300										

[a]Probabilities reflect a 200 g subsample and one analysis.

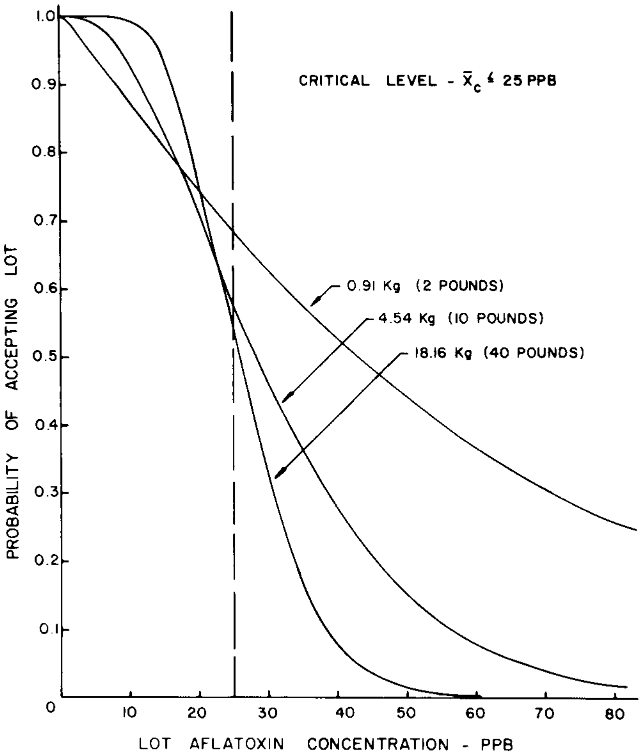

FIG. 4. Effect of sample size on the operating characteristic curve.

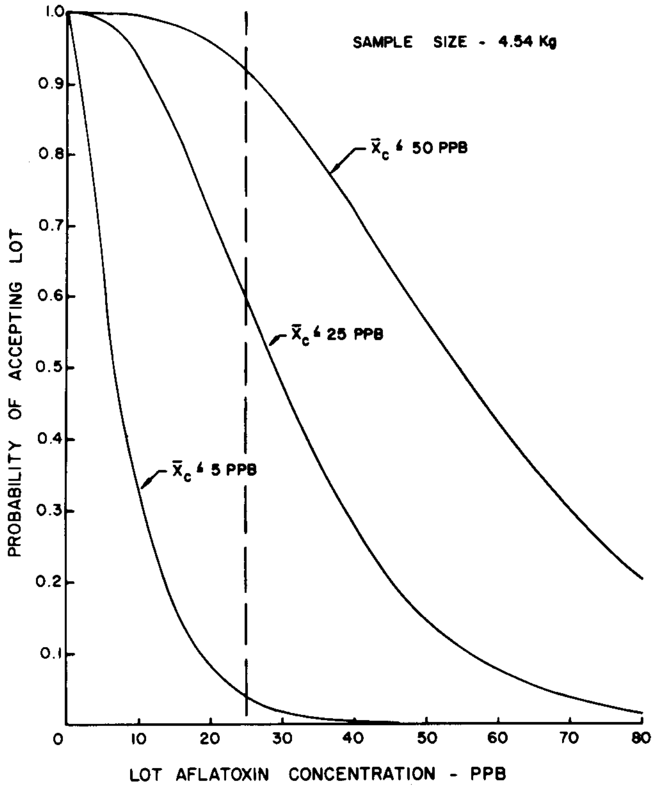

FIG. 5. Effect of the definition of good and bad sample quality on the operating characteristic curve.

μ and for a sample of size N_s. From the tables, OC curves can be constructed for various sample sizes N_s and for different definitions of good and bad sample quality (success level) $\bar{x}_c$.

The tables can also be used to show the effect of sample size and success level on the probability of accepting cottonseed lots.

Effect of Sample Size

The operating characteristic curves for three aflatoxin testing programs are shown in Figure 4. The sample size for the three testing programs are 0.91 kg (2 lb), 4.54 kg (10 lb), and 18.16 kg (40 lb) of dehulled cottonseed kernels. In all three cases, the aflatoxin in a 200 g subsample was extracted, and one 50 ml aliquot was analyzed for aflatoxin. When the test result is less than or equal to 25 ppb (success level), the lot is accepted.

The OC curves in Figure 4 show the acceptance probabilities for cottonseed lots at various aflatoxin concentrations when the three testing programs are used. As sample size increases, the probability of accepting lots at high concentrations decreases while the probability of accepting lots with low levels of aflatoxin increases. Increasing the sample size increases the slope of the OC curve about the success level which decreases both the consumer's and processor's risks. For example, when the sample size is increased from 0.91 to 18.16 kg, the acceptance probability for a cottonseed lot with 50 ppb decreases from 44.5% to 1.4% while the acceptance probability for a cottonseed lot with 10 ppb increases from 87.5% to 99%.

Effect of Changing the Definition of Good and Bad Sample Quality

Figure 5 shows the operating characteristic curves for the testing of a 4.54 kg sample when the success level or the definition of good and bad sample quality ($\overline{x}_c$) is varied from 50 to 25 to 5 ppb. Decreasing the $\overline{x}_c$ lowers the acceptance probability for all lot concentrations except 0 ppb for which the probability is always 100%. The primary disadvantage of lowering $\overline{x}_c$ to reduce the probability of accepting lots with high levels of aflatoxin is that the probability of accepting lots with low levels of aflatoxin decreases.

The effects of sample size and the definition of good and bad sample quality on the probability of accepting lots are demonstrated. The variability of cottonseed aflatoxin test results coupled with theoretical distributions are used to predict the probability of obtaining a cottonseed aflatoxin test result for a given lot concentration, sample size, subsample size, and number of analyses. These probabilities for various lot concentrations and sample sizes are provided to enable the reader to evaluate the consumer's and processor's risks associated with a cottonseed aflatoxin testing program.

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